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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "UV-Mutability and Genetic Recombination of Uv-Induced Gamma-Ray Sensitive Mutants of Neurospora crassa" submitted by Ram Deva Mehta in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

# ABSTRACT

Six gamma-ray sensitive mutants have been isolated in Neurospora crassa by means of uv-induction of conidia of a wild type strain pe (Y 8743m). All of these mutants showed different degrees of gamma-ray sensitivity. Genetic analysis of these mutants indicated that gamma-ray sensitivity of each of the mutant strain is controlled by a single nuclear gene. Tests for uv-sensitivity revealed that the gamma-ray sensitive mutants gs-6 is in addition sensitive to uv-radiation, whereas the five other mutants and their wild type progenitor are uv-resistant. Tests for uv-mutability showed that fewer mutations for caffeine resistance are induced in all the six gamma-ray sensitive mutants when compared to the wild type strain at equal levels of uv-dose or survival. Mutant gs-4 and gs-6 yielded a reduction in the frequency of uv-induced back mutations for adenine prototrophy. In homozygous condition none of the four mutations, gs-2, gs-4, gs-6 and gs-20 affected interallelic recombination as measured by the frequency of prototrophs ($pan^+$) resulting from pan-2(B3) x pan-2(B5) crosses.

The results discussed in the light of comparable data reported for prokaryotes and other eukaryotes facilitate the conclusion that in N. crassa the process of uv-mutagenesis is related to the dark repair systems and that the process of genetic recombination is not directly related to that of uv-mutability.

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# INTRODUCTION

Genetical and biochemical studies of radiation-sensitive mutants of Escherichia coli and its phages have established the fact that genetic recombination and uv-induced mutagenesis are related through the processes involved in the repair of DNA damaged by the treatment with radiation or chemical agents (Howard-Flanders, 1968).

Two types of dark repair mechanisms which are known in E. coli promote survival after uv-irradiation i.e., excision repair and post-replication repair or recombinational repair. Excision repair operates on the uv-induced DNA-lesions (mainly pyrimidine dimers) in such a manner that the lesions are enzymatically removed and the resulting gaps are replaced by the correct base sequence(Setlow and Carrier,1964; Boyce and Howard-Flanders,1964). Recombinational repair on the other hand, acts when the DNA containing dimers replicate and gaps are left opposite each pyrimidine dimer in the new DNA strand (Rupp and Howard-Flanders, 1968). DNA damage caused by ionizing radiation (X-rays or gamma-rays) and several chemical mutagens is probably repairable by the same dark repair mechanisms as mentioned above (Strauss, 1968).

Besides sensitivity to radiation and/or chemical mutagens, mutants defective in excision repair or recombinational repair are abnormal in their response to uv-mutagenesis and genetic recombination (Witkin, 1969). The properties of E. coli mutants with known biochemical defects in the repair processes have served as a basis for characterization of radiation sensitive mutants in eukaryotic micro-organisms in which studies at the biochemical level are lacking due to the difficulty encountered in specific radioactive labeling of the DNA (Cox and Parry, 1968; Davies, 1967; Grivell and Jackson, 1968).

The presence of DNA repair processes in eukaryotes has been demonstrated in various micro-organisms such as yeast and filamentous fungi (Cox and Parry, 1968; Resnick, 1969; Nakai, 1969; Kilbey and Smith, 1969; Fortuin, 1971a; Holliday, 1967; Davies, 1967; Schroeder, 1970).

Studies of radiation sensitive mutants of both bacteria and yeast, have revealed that mutants isolated primarily for their sensitivity to ionizing radiation commonly affect genetic recombination and uv-induced mutability (Howard-Flanders and Theriot, 1966; Nakai, 1969). Conversely, mutants isolated for recombination deficiency or for the loss of uv-induced mutability have been reported to be sensitive to uv and/or ionizing radiation (Clark and Margulies, 1965; Rodarte-Ramon and Mortimer, 1972; Lemontt, 1971a). The possibility thus exists that DNA repair of damage caused by ionizing radiation (X-ray or gamma rays) may have steps in common with the processes of uv-induced mutability and genetic recombination. With this view in mind, the present studies involving the isolation and characterization of gamma ray sensitive mutants of Neurospora crassa, have been carried out. In addition, an attempt has been made to study the effects of genes controlling radiation sensitivity on uv-induced mutagenesis and genetic recombination. As far as can be assessed, little is known of radiation sensitive mutants in Neurospora crassa, and for this fungus mutants isolated for their sensitivity to ionizing radiation have not yet been reported.

# REVIEW OF LITERATURE

Isolation of mutants sensitive to ultraviolet (uv) light and ionizing radiation and subsequent genetic studies, in both prokaryotes and eukaryotes, have proven that sensitivity to radiation is under genetic control (see review: Adler, 1966; Strauss, 1968).

PROKARYOTES: Among the prokaryotes, most of the studies pertain to various strains of Escherichia coli and its phages. Hill (1958) reported initially on the isolation of several mutants exhibiting increased sensitivity to the killing effects of uv-irradiation as compared to their parental strain, E. coli B. The isolation of some uv-resistant E. coli B mutants had been reported earlier by Witkin (1947). Concurrently, Luria (1949) produced evidence that uv-sensitivity in bacteriophages T2 and T4 was under genetic control and more recent studies (Rupert and Harm, 1966) indicated that radiation sensitivity of many other bacteriophages is intimately related to the properties of the host bacteria.

Following these observations, several workers have reported on the isolation of uv-sensitive E. coli mutants and attempts have been made to map the genes controlling radiation sensitivity on the bacterial 'chromosome'(Howard-Flanders et al., 1962; Rorsch et al.,1963; Van de Putte et al., 1963; Greenberg, 1964).

Biochemical evidence as to the basis of uv-sensitivity in bacteria is considerable. Exposure of E. coli to ultraviolet light causes the production of pyrimidine dimers (products in which neighbouring pyrimidines in the same strand of DNA are covalently linked) (Wacker et al., 1962). Almost an equal number of pyrimidine dimers are produced in the DNA of both wild type (uv-resistant) and uv-sensitive strains of E. coli when the cells are exposed to uv-irradiation (Setlow and Carrier, 1964; Boyce and Howard-

Flanders, 1964). The above investigators also established that when E. coli bacteria are incubated in the dark following uv-irradiation, pyrimidine dimers are released from the DNA of wild type cells, whereas uv-sensitive mutants are unable to eliminate them. They suggested that excision of pyrimidine dimers is part of a DNA repair process involved in the recovery of the cells after uv-irradiation. Not only in E. coli but also in T4 phage (Harm, 1964) genes have been discovered which control uv-sensitivity, probably by excision of pyrimidine dimers (Setlow, 1966).

DNA repair is assumed to involve a sequential action of various enzymes in such a way that the excision of pyrimidine dimers is followed by a repair synthesis resulting in the restoration of the normal DNA duplex (Howard-Flanders, 1968). There is biochemical evidence that excision of dimers causes single strand DNA breaks (Setlow et al., 1967) and the excised regions may be repaired by a repair replication mechanism (Pettijohn and Hanawalt, 1964).

At least three genetic loci(designated as uvrA, uvrB and uvrC) involved in the control of prymidine dimer excision in E. coli (Van de Putte et al., 1965; Howard-Flanders et al.,1966) are now known. The excision defective mutants are phenotypically designated as 'hcr$^-$' as these mutants are unable to carry out 'host cell reactivation' (i.e., the ability of the bacterial cell to reactivate or repair the uv damaged DNA of its bacteriophage (Harm, 1963)). In addition to host cell reactivation, the phenomenon called 'liquid holding recovery' (LHR), i.e., the capacity to recover in the dark when irradiated cells are incubated in a non-nutrient medium (usually a buffer) is also associated with excision repair (Harm, 1968). Excision defective mutants, unlike wild type, are unable to show liquid

holding recovery.

Besides being uv-sensitive, hcr⁻ strains are also sensitive to various chemical agens e.g., mitomycin c, nitrogen mustard (a bi-functional alkylating agent) and nitrous acid (Howard-Flanders et al., 1966; Howard Flanders and Boyce, 1966), all of which are known to cause predominantly inter-strand crosslinks in DNA (Howard-Flanders et al., loc. cit.). Hanawalt and Haynes (1965) as well as Howard-Flanders et al., (loc. cit.) proposed that the excision-repair system is not specific for uv-induced photo-products but that it is also involved in removing alterations produced by chemical agents.

Apart from the process of excision repair, which is not dependent on visible light and therefore constitutes a part of the dark repair mechanism, there is another process which is able to reverse the lethal effects of uv-light by post-irradiation treatment with visible light (Kelner, 1949). This phenomenon called photreactivation is under the control of a 'photoreactivating enzyme' which seems to interact with DNA containing uv-induced pyrimidine dimers by splitting them apart (Wulff and Rupert, 1962). Harm and Hillebrandt(1962) provided the first genetic evidence for the existence of an enzyme mediated photoreactivation process by isolating a mutant (phr⁻) from E. coli B which is unable to recover colony forming ability under illumination and therefore lacks the ability of the 'photoreactivating enzyme' present in the wild type (phr⁺) strain. Unlike excision repair, photoreactivation is specific for pyrimidine dimers only (Setlow, 1966).

UV-sensitivity is not only associated with the excision repair process but also with the process of genetic recombination (Clark and Margulies, 1965). These authors isolated E. coli mutants which were highly sensitive to uv-irradiation and in addition were unable to yield recombinants when tested in suitable crosses.

Recently several workers have reported on the isolation of recombination deficient (rec⁻) mutants of E. coli (Howard-Flanders and Theriot, 1966; Van de Putte, et al., 1966;Ogawa, Shimada and Tomizawa,1968; Emmerson, 1968), and of phages T4 (Rupert and Harm, 1966) and lambda (Echols and Gingery, 1968; Signer and Weil, 1968).

In E. coli recombination defective mutants have been detected either on the basis of their inability or reduced ability to form recombinants in a cross with an Hfr strain or on the basis of their uv-or X-ray sensitivity. All recombination deficient mutants tested so far, have been found sensitive to uv-light, ionizing radiation (X-rays or gamma-rays) and methyl methane sulfonate (MMS) regardless of the criterion used for their isolation (Howard-Flanders, 1968; Strauss, 1968). It should be noted that both X-rays and MMS are known to cause single strand breaks in DNA (Strauss, loc. cit.).

Genetic studies have shown that there are at least three different loci, recA, recB and recC which control genetic recombination (Emmerson, 1968; Willetts, et al., 1969). Mutations mapping at a different locus designated 'exr' cause increased sensitivity to uv, X-ray and nitrosoguanidine and exhibit reduced genetic recombination (Witkin,1969). Unlike excision defective mutants, rec⁻ and perhaps exr⁻ mutants can excise pyrimidine dimers from their DNA and have normal

host cell reactivation capacity (Clark et al., 1966; Witkin, 1969). hcr⁻ mutants differ from rec⁻ or exr⁻ mutants, in that hcr⁻ mutants do not exhibit any sensitivity to X-rays and perhaps to MMS (Howard-Flanders and Boyce, loc. cit.). However an exception to the above characterization was noted by Ogawa et al., (1968) who described E. coli mutants, mapping at a locus called uvr-D, which were phenotypically hcr⁻ but sensitive to uv-and gamma-irradiation. Apart from genetical studies, biochemical information shows that DNA-damage induced by X-rays, MMS and bifunctional alkylating agents is also repairable (Strauss, loc. cit.).

The two processes: excision repair and genetic recombination repair, appear to be independent and complementary to each other, as has been demonstrated by the fact that a double mutant carrying rec⁻ and uvr⁻ mutations is more sensitive than either of the parental strains (Howard-Flanders and Boyce, loc. cit.).

The biochemical nature of the defect in recombination deficient mutations is not as yet clearly understood, but there is an indication that these mutants are probably defective in a new type of dark repair process called 'post-replication repair' or 'recombinational repair' as suggested by Rupp and Howard-Flanders (1968). Their findings show that this repair process seems to operate on the daughter strand gaps which are produced when the DNA containing pyrimidine dimers replicates. Both wild type and excision defective strains are capable of repairing such gaps whereas rec⁻ mutants are unable to survive if their DNA (containing dimers) is allowed to replicate (Howard-Flanders, loc. cit.). Further support for the presence of "post-replication repair" and its independence of the excision repair process, was obtained by Radman et al.,

(1970). These authors concluded that excision repair is effective before DNA replication whereas recombinational repair occurs after and/or during replication. Their observations are based on survival studies of uv-irradiated phage λ on excision deficient (*uvr*$^-$A) and recombination deficient (*rec*$^-$) mutants of *E. coli* under conditions of normal or inhibited DNA replication. Studies on radiation sensitive mutants have revealed that DNA repair and uv-mutagenesis are inter-related. Recently these studies have been reviewed by Witkin (1969) and her conclusions and those of several other workers can be summarized as follows.

Mutations, from auxotrophy to prototrophy, to streptomycin resistance and to the inability to ferment lactose, are induced with a much higher frequency at low doses of uv in *hcr*$^-$ strains lacking detectable excision ability than in wild type *hcr*$^+$ strains. Similar results are obtained when uv-induced mutations are studied in *hcr*$^+$ strains post treated with inhibitors of the excision repair process such as caffeine and acriflavine. These results have been taken to indicate that unexcised pyrimidine dimers are responsible for most of the uv-induced mutations in *E. coli* and hence the excision repair process does not seem to be involved in uv-mutagenesis.

Both *rec*$^-$ or *exr*$^-$ mutations (which are defective in genetic recombination and are radiation sensitive) produce none or a very low frequency of uv-induced mutations as compared to their wild type (*rec*$^+$ or *exr*$^+$) counterparts. On the basis of these results Witkin postulates that the process of mutation induction by uv-light is, for the most part, similar if not indentical, to the process of genetical recombination and hence is related to the recombinational repair process.

Besides the low incidence of uv-induced mutability, mutations induced by gamma-rays or X-rays have been found to occur at a considerably lower frequency in some *exr*$^-$ or *rec*$^-$ mutants when compared with wild type strains (Bridges, Law and Munson, 1968; Kondo, 1968), suggesting a common mutagenic pathway for uv-light and ionizing radiation.

Contrary to Witkin's observations, a few *rec*$^-$ mutants and probably also some *exr*$^-$ mutants have been found to exhibit a response to uv-induced mutability comparable to wild type (Miura and Tomizawa, 1970; Kato and Kondo, 1967 and consequently these data seem to argue against a close relationship between uv-mutagenesis and recombination.

Biochemical studies on radiation sensitive mutants are very limited and have been largely unsuccessful in demonstrating the activity of enzymes involved in DNA repair processes. However, the finding that some mutants of *E. coli* as well as of T4 bacteriophage are radiation sensitive and at the same time are lacking the activity of certain enzymes e.g., DNA polymerase or polynucleotide ligase, is of particular interest (Dean and Pauling, 1970; Baldy, 1968). Recent reports indicate that the recombination-less strains *recB* and *recC* of *E. coli* lack an ATP-dependent endonuclease (Oishi, 1969; Barbour and Clar, 1970).

It should be mentioned that not only defective DNA repair processes (which are always responsible for increased radiation sensitivity) but also the impairment of other cellular processes e.g., process of cell division are sometimes effective in reducing the colony forming ability of the irradiated cells (Adler, 1966).

Radiation sensitive mutants have also been reported in bacterial species other than *E. coli* e.g., *Salmonella typhimurium*, *Micrococcus*

lysodeikticus, Serratia marcescens, Hemophilus influenzae, Bacillus subtilis and Pseudomonas aeruginosa (Strauss, 1968). In many cases the properties of these mutants are reminiscent of their E. coli counter parts. However one exception should be noted: most of the recombination deficient mutants isolated in P. aeruginosa have been found to be normal in their radiation sensitivity (Holloway, 1966; Van de Putte and Holloway, 1968).

EUKARYOTES.

Yeast. To demonstrate the genetic basis of radiation sensitivity in eukaryotes, yeasts (mainly Saccharomyces cerevisiae) have been extensively studies. Laskowski(1960) indicated that homozygosity of the gene controlling mating type led to an increased sensitivity to ionizing radiation. Later Moustacchi (1965) reported the isolation of a mutant resistant to ionizing radiation.

Nakai and Matsumoto (1967) isolated the first three mutants sensitive to uv-irradiation. One of these mutants proved to be sensitive to X-rays. Moreover, a double mutant, carrying two mutations one for uv-sensitivity alone and the other causing sensitivity to uv and X-rays both, showed an increased sensitivity to radiation when compared with the individual single mutant strains. This observation led these authors to assume that the repair pathways for the damage caused by uv- or X-irradiation are partly independent. Subsequently, Snow (1967 reported six non-allelic uv-sensitive mutants of S. cerevisiae, all recessive to wild type. Four of these mutants were found to exhibit sensitivity to nitrous acid.

In an attempt to discover all the genes involved in controlling dark repair processes in yeast, Cox and Parry(1968) isolated 96

uv-sensitive mutations which fell into 22 complementation groups separable by recombination. All mutations were found to be recessive to wild type and five of these mutants exhibited an increased sensitivity to gamma-ray irradiation when compared to the wild type control strain. Zimmermann, (1968) tested the sensitivity of these mutants to nitrous acid ($HNO_2$) and methyl methane sulfonate and reported that most of the mutants sensitive to $HNO_2$ are also sensitive to MMS. Four of the mutants were sensitive to all the four agents i.e., uv, gamma-rays, MMS and $HNO_2$. Moreover the extent of cross sensitivity to various agents was variable. For instance a mutant which is highly sensitive to uv may be only slightly sensitive to gamma-rays. These observations led to the assumption that several independent pathways are involved in the cause of a given uv-induced damage and that each of these pathways may have different efficiency in repairing damage caused by other agents. Based upon their observations that uv-sensitive mutants are cross-sensitive to nitrogen mustard and X-ray sensitive mutants exhibit sensitivity to MMS inactivation, Brendel et al., (1970) have suggested the involvement of similar common steps in the repair of alkylation and radiation damage in yeast as were observed in bacteria (Strauss, loc. cit.).

Resnick, (1969) isolated mutants which showed sensitivity to either uv or X-ray or to both. Two of his X-ray-sensitive mutants (xs-2 and xs-3) turned out to be allelic to the intragenic recombination (mitotic) deficient mutants isolated by Rodarte-Ramon et al., (1968). The latter authors obtained 10 mutants deficient for mitotic intragenic recombination of which four proved to be X-ray-sensitive and thus provided evidence for the existence of a relationship between radiation sensitivity and genetic recombination in yeast. However, subsequent studies by

Rodarte-Ramon and Mortimer (1972) have suggested that some steps of the recombination process in yeast may be independent of the process of radiation damage repair since four of their recombination deficient mutants exhibited wild type response to both, x-ray and uv-light. Resnick (loc. cit.) obtained a uv-sensitive mutant, uvs$^{-}$9 which showed normal sensitivity to X-ray and exhibited a much higher uv-induced mutation frequency for all loci studied as compared to the wild type: a characteristic which resembles that of E. coli mutants defective in excision repair.

Double mutants carrying alleles, conferring sensitivity to uv-light or X-ray or both, in various combinations, were constructed by Khan, et al., (1970). A study of these mutants suggested that there exists at least three independent pathways for repairing DNA damaged by radiation: (i) a pathway which repairs uv-damage, (ii) a pathway which alters X-ray damage and (iii) a pathway capable of handling components of both uv- and X-ray damage. Existence of independent repair pathways as suggested by Khan, et al., (loc. cit.) is also implicit in the work of Brown and Kilbey (1970) who isolated 'hyper' uv-sensitive mutants from a uv-sensitive strain uvs-1 reported on by Nakai and Matsumoto (loc. cit.). Two of these hyper-sensitive mutants showed an increased sensitivity to gamma-rays.

Nakai (1969) reported that an X-ray sensitive mutant xs1 which is normal in its sensitivity to uv, exhibited a reduction in the frequency of somatic recombination and failed to form tetrads. The author claims that these characteristics resemble those of the rec$^{-}$ mutants of E. coli. However, unlike E. coli mutants, the xs1 mutant yielded a normal

or higher frequency of X-ray induced mutations when compared with the wild type strain at equal doses. An increased frequency of uv-induced mutations was observed in a uv-sensitive mutant uvs-1, which is normal in its sensitivity to X-rays (Nakai and Yamaguchi, 1969).

Kilbey and Smith (1969) argued that mutant uvs-1 (described above) resembled a bacterial excision defective mutant in its sensitivity to diepoxybutane (a bifunctional alkylating agent), nitrosoguanidine and in its responses to photoreactivation and liquid holding recovery.

Moustacchi and Enteric (1970) compared the survival of a uv-sensitive mutant (uvs1-3) with the wild type strain, in both haploid and diploid state, after post-irradiation treatment for photoreactivation and liquid holding recovery. On the basis of their observations, the authors suggest that there are at least two types of dark repair processes for altering uv-damage in yeast. One is active in haploid resting cells and is probably related to excision repair mechanism; whereas the other dark repair mechanism is active in dividing haploid cells or diploid cells either resting or dividing, may involve chromosomal exchanges. The latter mechanism appears to be equivalent to recombinational repair as postulated by Rupp and Howard-Flanders, (1968) for E. coli. Mutants sensitive to both uv and X-rays have been reported to show a variable response for uv-induced mutability (Averbeck et al., 1970). Two of the mutants, $r^s1$ and $r^s3$-2 showed increased uv-induced reversion frequemcy from auxotrophy to prototrophy as compared to the wild type strain. However, reduced frequency of uv-induced mutations was observed in mutant $r^s2$, whereas no revertants, induced or spontaneous, have been detected in mutant $r^s3$-1. It should be noted that both $r^s3$-1 and $r^s3$-2 are mutations of the same gene.

In yeast, uv-induced lesions, presumably pyrimidine dimers are responsible for most of the uv-induced mutations and for mitotic gene conversion (Parry and Cox, 1965, 1968; Pittman, 1961). The basis of these observations concerns the fact that (i) both, the frequency of gene conversion and the frequency of uv-induced mutations are positively correlated with increasing uv-dose and (ii) after photoreactivation treatment the effect of uv-irradiation is considerably reduced. Moreover, uv-sensitive mutants which are presumably defective in excision repair,yielded an increased frequency of uv-induced mutations as well as of uv-induced mitotic gene conversion when compared with the wild type strain of yeast (Mori and Nakai, 1968; Snow, 1968; Moustacchi, 1969; Nakai and Yamaguchi, 1969).

These studies, thus seem to indicate that uv-induced lesions stimulate genetic recombination or at least mitotic gene conversion. In E. coli similar observations have been made, which show that the frequency of uv-induced genetic recombination is considerably higher in hcr$^-$ strains when compared to wild type (hcr$^+$) strains at equal doses of radiation (Howard-Flanders and Boyce, 1966). On the other hand, Mori and Nakai (loc. cit.) observed that the frequency of uv-induced or X-ray induced mitotic gene conversion in X-ray sensitive mutants of S. cerevisiae was almost completely suppressed over the dose range investigated. Snow (1968) observed that the meiotic recombination in diploid uv-sensitive mutants did not significantly differ from that of wild type strains.

A new approach was made by Lemontt and Mortimer (1970) for studying the relationship between radiation sensitivity, uv-induced mutability and genetic recombination. These authors isolated mutants

of S. cerevisiae by direct selection for reduced uv-induced mutability. In testing, these mutants were found to be moderately sensitive to both uv and X-rays (Lemontt, 1971a) and are referred to as 'reversionless' (rev) mutants. None of the rev mutations tested, were found to affect either spontaneous meiotic intergenic recombination or radiation (uv and X-ray) induced mitotic intergenic recombination, suggesting that recombination events may not necessarily be essential for uv-mutagenesis.

Mutants defective in photoreactivation were isolated in S. cerevisiae by Resnick (1969). More direct evidence for the existence of a photoreactivation mechanism in yeast was presented by Rupert (1960) who extracted the photoreactivating enzyme.

Besides S. cerevisiae, uv-sensitive mutants have been developed in Schizosaccharomyces pombe (Fabre, 1971; Haefner and Howrey, 1967; Guglielminetti and Schupbach, 1968, Nasim, 1968). Nasim, (1968) observed one highly uv-sensitive mutant of S. pombe showing significantly reduced frequency of uv-induced (adenine auxotrophs) forward mutation and two mutants with intermediate uv-sensitivity exhibiting higher forward mutation frequency after uv-induction.

Not only mutations of nuclear genes can be held responsible for conferring radiation sensitivity: cytoplasmic mutants and mutations impairing the process of cell division as isolated in yeast, may have a similar effect (Nakai, 1969).

Other microorganisms.

In Aspergillus rugulosus mutants sensitive to uv and nitrous acid have been described by Lennox and Tuveson (1967) and in A. nidulans by Lanier et al., (1968). In A. nidulans crosses homozygous for mutations affecting radiation sensitivity were found to be sterile.

Recently six uv-sensitive mutants have been reported on in A. nidulans which fall into four complementation groups: uvsB, uvsC, uvsD and uvsE (Jansen, 1970a; Fortuin, 1971a). Two of these mutants, uvsC and uvsE are uv-sensitive only when germinating conidia of these strains are irradiated whereas dormant conidia of uvsB and uvsD mutants are uv-sensitive. Presuming that recombination repair only occurs in germinating conidia and excision repair in dormant conidia, Fortuin (1971b, 1971c) and Jansen (1970b) argued that mutants uvsC and uvsE are defective in recombination repair and uvSB and uvsD are defective in excision repair. Further studies showed that the uvsD mutation enhances, and the mutation uvsE diminishes spontaneous mitotic intragenic recombination (Fortuin 1971b, 1971d). On testing, the uvsE mutant showed an increased sensitivity to X-ray and in addition exhibited normal uv-induced mutability. The uvsD mutation on the other hand, showed a reduced frequency for uv-induced mutability. Crosses homozygous for uvsD or uvsE were found to be partially sterile and in these crosses no significant effect on meiotic recombination was observed. On the other hand, uv-sensitive mutants of A. nidulans are known, which exhibit an abnormally high frequency of spontaneous mitotic intergenic recombination (Shanfield and Käfer, 1969).

In Ustilago maydis, Holliday (1965) isolated three uv-sensitive mutants, uvs-1, uvs-2 and uvs-3. Both uvs-1 and uvs-2 showed an increased sensitivity to X-rays. Recombination studies indicated that meiotic recombination is normal in uvs-1 and uvs-3 whereas the mutation uvs-2 completely blocks meiosis (Holliday, 1967). uv-induced mitotic intergenic recombination is suppressed in both uvs-2 and uvs-3. In addition, mutant uvs-3, showed an increased mitotic gene conversion when compared to the wild type. An increased frequency of spontaneous mitotic recombination was observed in the mutant uvs-2.

Ultraviolet sensitive mutants have been reported in the green alga Chlamydomonas reinhardi by Davies (1967). Some of these mutants showed an increased uv-induced mutability when compared to the wild type strain (Davies and Levin, 1968).

In Neurospora crassa, Chang and Tuvesor (1967) published data on the isolation of two uv-sensitive mutants. Both of these mutants exhibited also an increased sensitivity to nitrous acid whereas one of these mutants, uvs-1 was also found to be sensitive to the treatment of nitrosoguanidine. Crosses homozygous for these mutations were sterile.

Stadler and Smith (1968) isolated in Neurospora crassa another uv-sensitive mutant (uvs-2) which in homozygous condition had no effect on meiotic recombination. Recently Schroeder (1970a,1970b) has reported on three uv-sensitive mutants , uvs-3, uvs-4 and uvs-5. Crosses homozygous for each of these mutations were either sterile or did not show any effect on meiotic recombination. Mutant uvs-3 showed an increased sensitivity to X-rays and nitrosoguanidine. In her conclusion Schroeder pointed out that defects in mutants uvs-3 were associated with meiotic abnormalities and perhaps with an increased frequency of mitotic recombination.

A mutant, upr-1 isolated in Neurospora crassa showed an increased sensitivity to uv-light in the dark and was found to be defective in photoreactivation (Tuveson and Mangan, 1970), indicating the existence of photoreactivating enzyme. Direct evidence for the existence of a photoreactivation process in N. crassa was already known from the work by Terry and Setlow (1967), who extracted the photoreactivating enzyme from this fungus.

Recently De Serres (1971) studied uv-induced mutability in all the uv-sensitive mutants of N. crassa described above. Both uvs-3 and uvs-4 showed a marked reduction in the frequency of uv-induced forward mutations

at the ad-3 A and ad-3 B loci, whereas uvs-2 was highly and uvs-1, upr-1, uvs-5 were less mutable as compared to wild type strain. Chang et al (1968) earlier reported that mutant uvs-1 also showed a reduced frequency of forward mutations for caffeine resistance when induced by uv. The fact that the characteristic to affect mutability is not a sole property of uv-sensitive mutants was shown by Mehta and Weijer (1971) ,who studied uv-induced mutability in gamma-ray sensitive mutants of Neurospora crassa.

As in yeast, a non-nuclear control for uv-sensitivity exists in N. crassa (Chang, Tuveson and Munroe, 1968).

Not only in microorganisms but also in higher plants (Riley and Miller, 1966) and animals (Watson, 1969) radiation sensitive mutants are known which are defective in meiotic recombination.

In summary, studies pertaining to the genetic control of radiation sensitivity in both, prokaryotes and eukaryotes as described above, appear to indicate an underlying relationship between the mechanisms involved in DNA repair, uv-induced mutagenesis and genetic recombination. Nevertheless, the relationship seem to be far more complex in eukaryotes than in bacteria and phages. This complexity at the eukaryotic level may reflect the complexities of the nature of DNA repair processes and also the complexities of processes of mutagenesis and genetic recombination in these different organisms.

# MATERIALS AND METHODS

## 1. Strains

The following strains of Neurospora crassa were used in this study:

(1) pe (Y8743m) a, wild type reference and the strain in which gamma-ray sensitive mutants were induced

(2) pe (Y8743m) A

(3) 46-5, al-2 (15300), pan-2 ($B_3$), tryp-2 (75001), A

(4) 467, ad-1 (3254), pan-2 ($B_5$), a

(5) fl (L) a ) Strains used as tester stocks for
(6) fl (L) A ) mating type determinations.

(7) uvS-3 a

The phenotypic characteristics associated with the alleles described above are as follows:

pe = Peach coloured conidia

al-2 = Albino conidia

pan-2 (B3) = A requirement for pantothenic acid; pan-2 (B3) complements with pan-2 (B5)

pan-2 (B5) = A requirement for pantothenic acid; pan-2 (B5) complements with pan-2 (B3)

tryp-2 = A requirement for tryptophan

ad-1 = A requirement for adenine

fl = Fluffy, little aerial growth and non-conidiating.

A and a = Mating type alleles

uvS-3 = Sensitive to uv and x-ray

The loci pe and fl are situated on linkage group II; the mating type (A vs. a) and al-2 loci are on linkage group I. The loci ad-1, pan-2 and tryp-2 belong to linkage group VI.

All the strains were obtained from the Fungal Genetic Stock Center (Hanover, New Ham except number 3 and 4 listed above, which were obtained from Dr. S.F.H. Threlkeld, McMaster University, Hamilton (Ontario). All the strains described above are known for their mixed genetic background ( arratt and Ogata, 1970).

2. MEDIA

Minimal medium. The strains were usually grown on solidified Vogel's Medium N (Vogel 1964) supplemented with the appropriate growth factors at the rate of 50 mg per litre of medium. For the growth curve studies liquid minimal medium (without solidifying component) was used.

Complete medium. Ascospores, from crosses segregating for biochemical markers, were isolated on solidified complete medium consisting of Vogel's Medium N plus 0.5% yeast extract, 0.5% casein hydrolysate, 0.1% standard vitamin solution and 0.005% tryptophan.

Sorbose medium. For single colony isolation or for colony counts, solidified sorbose media of the following compositions were used.

(i) Sorbose minimal medium. Medium N was supplemented with 1.5% sorbose, 0.05% glucose and 0.05% fructose (Brockman and de Serres, 1963). Where necessary an appropriate growth factor at the rate of 0.005% was added.

(ii) Sorbose complete medium. Medium N plus 0.5% yeast extract, 0.5% casein hydrolysate, 0.1% standard vitamin solution, 0.005% tryptophan, 1.5% sorbose, 0.05 glucose and 0.05% fructose

Crossing Medium. All crosses were made on synthetic crossing medium (Westergaard and Mitchell, 1947). Biochemical requirements were added, where necessary, at the rate of 50 mg/

litre of medium. All media were solidified with 1.5% agar.

3. METHODS

(i) Conidial suspensions: Conidia were harvested from 7-10 days old cultures growing on the slants in test tubes at 25°C, in sterile 0.066M phosphate buffer (Na-Na), pH7.0 (Chang and Tuveson, 1967). Five ml of buffer solution was added to each culture tube. After adding some glass beads, the tubes were shaken vigorously on a Vortex Mixer for about 2 minutes. This suspension was then filtered through sterile cheese cloth and glass wool twice with one more shaking on the Vortex mixer before second filteration. This operation yielded a conidial suspension free of mycelial fragments and clumps of conidia. The concentration of conidia in the suspension was determined by haemocytometer counts. Four samples of each suspension were counted and the average of these counts was used to calculate the concentration of the given suspension. Subsequently suspensions were adjusted to the required concentrations.

(ii) Gamma-irradiations: A gamma cell with a Cobalt-60 source of gamma rays was used for irradiation experiments. The gamma-dose-rate was measured by using a Fricke dosimeter (Allen, 1961).

Isolation of gamma-ray-sensitive mutants. The gamma-ray sensitive mutants were induced in pe (Y8743m) a with uv light (uv dose: 2700 ergs $mm^{-2}$, dose rate: 10 ergs $mm^{-2}$ $sec^{-1}$). uv-irradiated conidia of this strain were

plated on sorbose minimal medium (approximately 70 viable conidia /plate) and incubated in the dark at 25°C for 48-50 hours. Replica plating methods were employed using filter paper saturated with minimal medium supplemented with sorbose, fructose and glucose. The filter papers bearing the growing colonies were placed on solidified minimal medium (supplemented with sorbose, fructose and glucose) petri plates and were exposed to gamma-rays (dose 67 Krads; dose rate: 6.7 Krads ($min^{-1}$) from a 60-cobalt source. After irradiation they were incubated at 25°C. After 3 days of incubation absence of colonies or very limited growth of colonies on the replica plates (when compared with the "master" plates) was scored and subsequently these colonies were isolated from the "master" plates as presumptive gamma-ray sensitive mutants. Twenty suspected gamma-ray mutants were isolated in this manner from 7250 surviving colonies. All the twenty-isolates were then retested for gamma-ray sensitivity and only 8 isolates turned out to be gamma-sensitive. On subculturing for 10-15 times, two of the eight mutant reverted back to the wild type phenotype. The remaining 6 mutants were back-crossed to the wild type pe ($Y8743^m$)A and recovered by single ascospore isolation from these crosses. These six mutants were designated as, gs-1, gs-2, gs-3, gs-4, gs-6 and gs-20.

Gamma-ray survival curves: Four ml of the conidial suspension ($1 \times 10^6$ conidia/ml) of a given strain were delivered into each of seven sterile tubes (75 x 11 mm). One tube was kept

as control and the remaining six were irradiated simultaneously at isodose positions in the Cobalt-60 gamma cell. Each tube was then taken out of the cell at the predetermined time interval. After making appropriate dilutions, control and irradiated samples of conidia were plated on sorbose minimal medium and incubated at 30°C for three days before colonies were counted. The dilutions were so adjusted that, depending upon the dose of radiation used, each plate received $5 \times 10^2 - 10^4$ conidia of wild type and $10^3 - 3 \times 10^4$ conidia of gamma-ray sensitive mutants. The control plates in all the strains received only $10^2$ per plate as to minimize the difference in the colony density per plate under various treatments of different strains.

Three independent experiments were carried out for each mutant strain and wild type. Five plates were used for each radiation level. Each mutant strain was irradiated simultaneously with wild type as a control.

Qualitative tests for gamma-ray sensitivity: Qualitative tests for gamma-sensitivity were carried out by irradiating a 4 ml conidial suspension ($1 \times 10^5$ conidia/ml) with a dose of 134 krads. The tubes were properly shaken and three drops of each irradiated sample were placed separately on the sorbose medium with the tip of 1 ml pipette and incubated at 30°C. After 3 days of incubation gamma-ray sensitivity was indicated either by an absence of growth or by extremely poor growth. The wild type strain was always used as the control reference in gamma-ray sensitivity tests.

(iii) uv-irradiation:

A General Electric G 15T8 Germicidal lamp (15 Watt), which transmits about 50% of its energy at a single wavelength of 2537Å, was used as a source of uv-irradiation. The uv-dose-rate was measured by chemical actinometry using an uranyl oxalate solution (Calvert and Pitts, 1967).
For uv-survival curves and for mutation induction, a 15 ml sample of a conidial suspension ($1 \times 10^7$ - $1 \times 10^8$ conidia/ml) from each of the gamma-ray sensitive mutants and the wild type strain, were irradiated with uv-light in 10 cm glass petri dishes. During irradiation the samples were gently agitated at a fairly constant (22.5°C) temperature.

For the induction of forward mutations for caffeine resistance or acriflavine resistance, a conidial suspension of a particular strain was irradiated with various doses of uv-light (dose rate 11.3 ergs $mm^{-2}$ $sec^{-1}$). One ml sample was withdrawn after each dose treatment (time-interval) and subsequently diluted in sterile distilled water to predetermined conidial density levels.

Diluted samples of irradiated and unirradiated conidia were plated on sorbose minimal medium either supplemented with caffeine (2500 µg/ml) or acriflavine (10 µg/ml). Each plate was spread with $1 \times 10^6$ - $1 \times 10^7$ conidia. For the determination of survival counts sorbose minimal medium plates were used. These plates received $10^2$ - $10^4$ conidia/plate

depending upon the level of the uv-dose. All plates were incubated in the dark at 25°C. uv-irradiation and subsequent plating of the samples were performed under yellow light to prevent photoreactivation.

Plates for viability and mutability tests were incubated for 4 and7 days respectively. Due to the fact that the concentrations of caffeine or acriflavine employed in the medium do not permit the growth of wild type conidia, any colonies, regardless of the rate of growth, appearing on the plates supplemented with caffeine or acriflavine, were considered as caffeine or acriflavine resistant mutants.

The mutation frequency for caffeine or acriflavine resistance as well as uv-survival frequencies for all the strains were calculated on the basis of the pooled data of the three independent experiments. Three plates were used for each uv-dose level and for the control.

For the induction of ad+ revertants, unirradiated and irradiated samples of conidia were spread on plates of sorbose minimal medium supplemented with the appropriate growth factors but adenine. The induction procedure for ad+ revertants is similar to the one described above for the induction of caffeine resistant mutations.

IV. Post uv-irradiation Treatment:

Fifteen ml of conidial suspension ($1x10^7$/ml) were irradiated with uv-light (dose: 678, 2034, 3390 ergs $mm^{-2}$, dose rate 11.3 ergs $mm^{-2}sec.^{-1}$). One ml sample of conidial suspension was withdrawn after each dose treatment and 0.1 ml of this sample was put into each of the 4 tubes containing 5 ml of sterile distilled water and into each of the two tubes containing 5 ml of caffeine (.25% in distilled water) solution. Unirradiated samples were similarly treated One set of 4 tubes each (3 tubes, containing samples irradiated with three different uv-doses and 1 tube containing unirradiated sample) was subjected to photoreactivation, delayed photoreactivation, liquid holding and immediate plating. The set of the tubes containing caffeine solution were used to study the effect of caffeine on delayed photoreactivation and liquid holding.

Photoreactivation:

For photoreactiviation, tubes were put at a slant on a wire mesh which in turn was placed between 4 General Electric 40 Watt, 47 inch cool white fluorescent lamps ; two in a fixture 15 cm above and two 20 cm below the tubes. A fan was placed horizontally at one end of the tubes to circulate the air between the lamps so as to maintain the temperature at 25°C. A 30 minute exposure was used as the standard photoreactivation time as this treatment caused maximum photoreactivation of the wild type strain irradiated with a uv-dose of 3390 ergs $mm^{-2}$.

Delayed photoreactivation:

To test delayed photoreactivation ability both in presence or absence of caffeine, conidial suspensions were held in the dark for 6 hours at 25°C followed by a 30 minute photoreactivation treatment.

Liquid Holding:

Samples with and without caffeine were held in the dark for 6 hours at 25°C. After these treatments conidial suspensions were further diluted and plated on sorbose minimal medium. For survival counts (immediate plating) samples were diluted and plated on sorbose minimal medium immediately after irradiation.

All plates were incubated in the dark at 25°C for 4-5 days before the colonies were counted.

V. Mating, isolation of unordered asci and random ascospores.

In each cross a protoperithecial strain was grown in petri-dishes each containing 15 ml of synthetic crossing medium with appropriate supplements for 5-6 days at 25°C. At the end of the incubation period excessive mycelia were removed from these plates. For fertilization one ml of conidial suspension ($1x10^6$/ml) from 7-8 days old cultures of each of the strains used as spermatial parents, was distributed over the surface of the plates of the respective protoperithecial parent. The plates were further incubated for a period of 15-20 days. Removal of excessive mycelia ensured the formation of greater number of perithecia in the plates and the release of a larger quantity of ascospores on the lid of the crossing plates.

Unordered tetrads were collected as shot groups of ascospores on 4% agar-water slabs placed on microscope slides under the inverted cross plate (Strickland, 1960). Spores were isolated in individual 75 mm tubes containing 2.5 ml of solidified complete medium which were heat-shocked for 30 minutes in a 60°C water bath and incubated at 25°C. After 5-6 days of incubation, cultures were stored at 4°C until used for

further tests.

To collect the random ascospores from the crossing plates the method as described by Fincham (1967) was applied. After heat-shock treatment (as described above) ascospore suspensions were diluted as required and spread on the surface of the solidified sorbose medium supplemented with appropriate biochemical requirements. The plates were incubated at 25°C for 5-6 days. At the end of the incubation period plates were stored at 4°C until these were scored for viable counts and phenotypic characterization of the colonies.

VI. Genetic Analysis:

The scoring for genetic segregation of gamma-ray sensitivity (gs) was done in the following crosses in which strain 46-5, al-2 pan-2 (B3), tryp-2A, was used either as the protoperithecial or spermatial parent:

gs-1 pe a x 46-5 al-2 pan-2 (B3), tryp-2 A

gs-2 pe, a x 46-5, al-2 pan-2 (B3), tryp-2 A

gs-3 pe a x 46-5 al-2 pan-2 (B3), tryp-2 A

gs-4 pe a x 46-5 al-2 pan-2 (B3), tryp-2 A

gs-6 pe a x 46-5 al-2 pan-2 (B3), tryp-2 A

gs-20 pe a x 46-5 al-2 pan-2 (B3), tryp-2 A

The reciprocal cross 46-5, al-2 pan-2 (B3), tryp-2 A x gs-3 pe a could not be established due to male sterility of the strain gs-3 pe a.

Six to ten complete tetrads resulting from each cross (where possible including the reciprocal cross) were analyzed for the segregation of genetic markers, mating type (A/a), pan-2, tryp-2 and gs. Scoring for the segregation of gs with respect to marker pe was not possible.

difficulty in differentiating the peach phenotype from the wild type conidial colour.

One unordered tetrad resulting from a cross wild type, pe (Y8743m)a x 46-5 al-2 pan-2 (B3) tryp-2 A was used as a control in the qualitative tests performed for the segregation of gamma-ray sensitivity. An individual ascospore culture was streaked on sorbose medium, sorbose minimal medium plus pantothenic acid or tryptophan or both to score the phenotype of the segregants with respect to the biochemical marker pan-2 and tryp-2. Mating type tests were performed by placing a drop of conidial suspension on the plates of tester stocks, fl a and fl A.

VII. Inter allelic Recombination Frequency:

The frequency of recombination between two pan-2 alleles, pan-2 (B3) and pan-2 (B5) was studied in the following reciprocal crosses:

(1) 46-5, $gs^+$, al-2, pan-2 (B3), tryp-2A x 467, $gs^+$, pan-2(B5), ad-1 a

(2) $gs^+$, al-2, pan-2 (B3), tryp-2, a x $gs^+$, pan-2 (B5), ad-1 A

(3) gs-2, pan-2,(B3) tryp-2 a x gs-2, pan-2(B5), ad-1 A

(4) gs-4, pan-2, (B3) tryp-2 a x gs-4, pan-2 (B5,) ad-1 A

(5) gs-6, al-2, pan-2 (B3), tryp-2 a x gs-6, pan-2 (B5) ad-1 A

(6) gs-20, pan-2(B3), tryp-2 A x gs-20, pan-2 (B5), ad-1 a

The above strains (involved in crosses 2-6) were obtained by crossing the gamma-ray sensitive mutants (gs-2, gs-4, gs-6, gs-20) as well as their wild type progenitor $gs^+$pe (Y8743m) individually with 46-5, al-2, pan-2 (B3) tryp-2 A and with 467, ad-1, pan-2(B5) a. Before these strains were used for recombination studies (as indicated above), all of them were back-crossed twice to wild type pe (Y8743m) to minimize the background genetic differences between them.

To determine the frequency of *pan*$^+$ recombinants between *pan*-2 (B3) and *pan*-2 (B5), each cross was made 4 times. To select *pan*$^+$ recombinant colonies, random ascospores originating from each of the crosses were heat-shocked and plated on 17-18 sorbose minimal medium plates supplemented with adenine and tryptophan. Each plate contained approximately $1x10^5$ ascospores. For ascospore viability counts three sorbose minimal medium plates supplemented with tryptophan, adenine and calcium pantothenate were used for each cross. Each plate was spread with heat-shocked ascospore suspension containing approxiamtely 100 spores. The plates were incubated at 25°C and colonies were counted after 4-5 days and results were recorded. Ascospore viability varied from 50 - 80% in these crosses.

VIII. Nuclear Numbers:

Samples of conidia from wild type and gamma-ray sensitive mutants (7-8 days old cultures) were stained with aceto-orcein following the procedure used by Barry, (1966). Number of nuclei included in each conidium were counted under the microscope. On an average about 600-800 conidia were counted from each strain. Conidia which showed no nuclei were considered unstained and were not counted. All the conidia in each observed field were counted.

IX. Growth Studies:

Ten ml of the conidial suspension ($1 \times 10^6$ conidia/ml) was delivered into a flask containing 100 ml of liquid minimal medium. One flask was used for each time interval. The flasks were kept on a reciprocating shaker at 25°C. At the end of each predetermined time

interval, contents of the respective flasks were filtered through milipore filters or glass filters. The filtered samples were dried over-night under vacuum in a desiccator and the dry-weight of mycelia was determined.

X. Electron Microscopy:

Four ml samples of conidia (density; $5 \times 10^7$ conidia/ml) were irradiated with gamma-rays (dose: 31 Krads, 93 Krads, 186 Krads). Both irradiated and unirradiated samples of conidia were subjected to electron microscopic studies. For ultra-structural studies of mycelia the controlled and irradiated conidia were plated on solidified minimal medium and incubated at 25°C. After 24 hours of incubation, mycelia were removed from the surface of the medium by means of scraping.

Conidia and mycelia were fixed in 3% gluteraldehyde in 0.1 M phosphate buffer for eight hours (see Pease, 1964). After a brief rinse in phosphate buffer the material was post fixed in 2% osmium tetraoxide, (prepared in 0.1 M phosphate buffer) for 4 hours. The fixed material was dehydrated in grades of ethanol. The fixation and dehydration were carried out at room temperature. The mycelia and conidia were embedded in Arldite. Thin sections were cut with a diamond knife on a Reichert Ultra-microtome and picked up on Formvar coated 75 mesh grids. The sections were stained in uranyl acetate and lead citrate. The stained sections were examined in a Philips EM300 electron microscope at 60 KV.

XI. Statistical Analysis:

Since the number of mutants observed were limited, the significance of each observed value was expressed with the limits of probability of a mutational event corresponding to the 95% probability levels (Stevens, 1942).

FIG. 1. Gamma-ray survival curves for a wild type strain, pe (Y8743m)a and three gamma-ray sensitive mutants, gs-1, gs-2, and gs-4 of Neurospora crassa. The survival curves represent the average of three independent experiments. Doserate: 6.7 Krads $min^{-1}$.

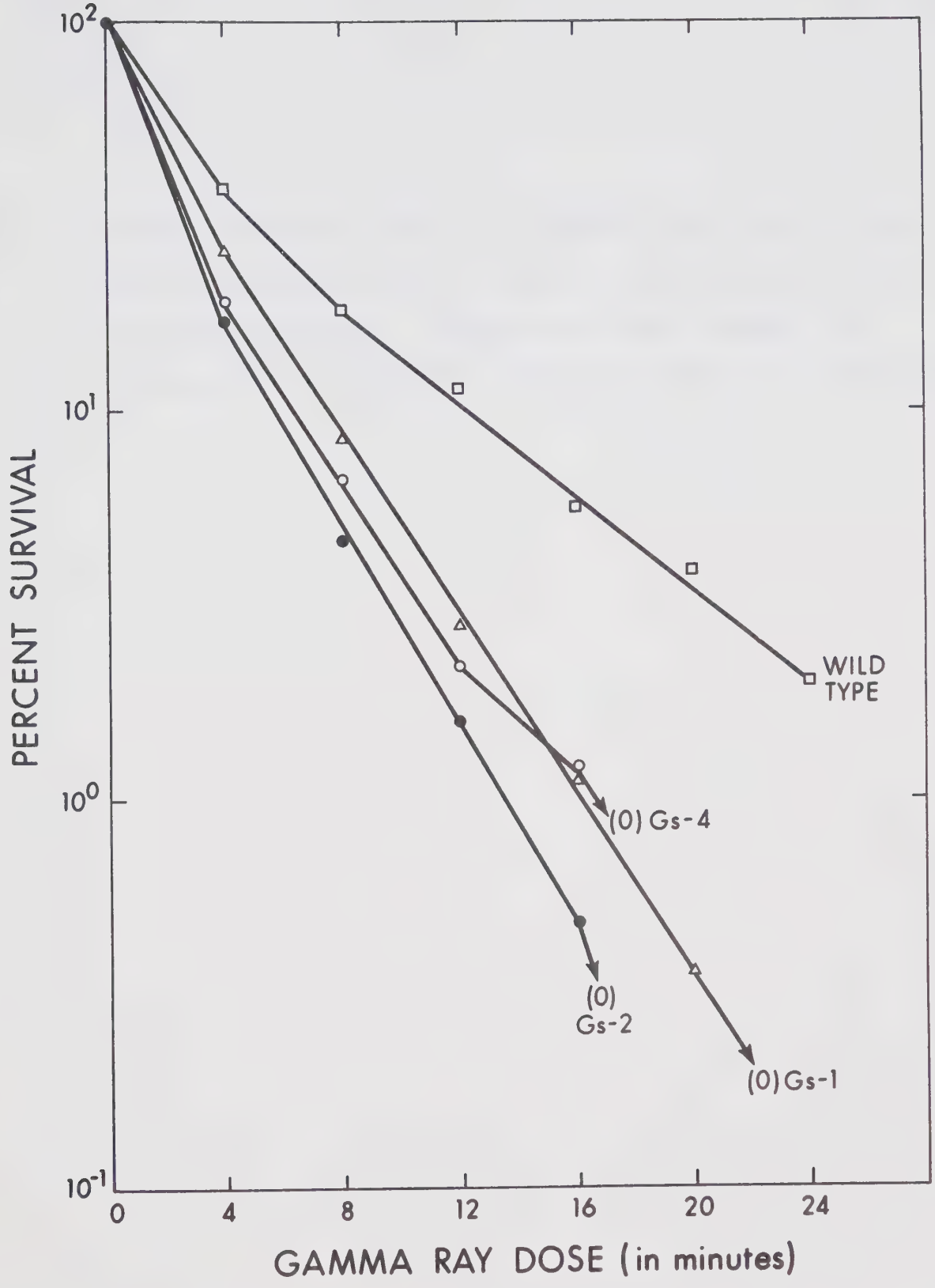
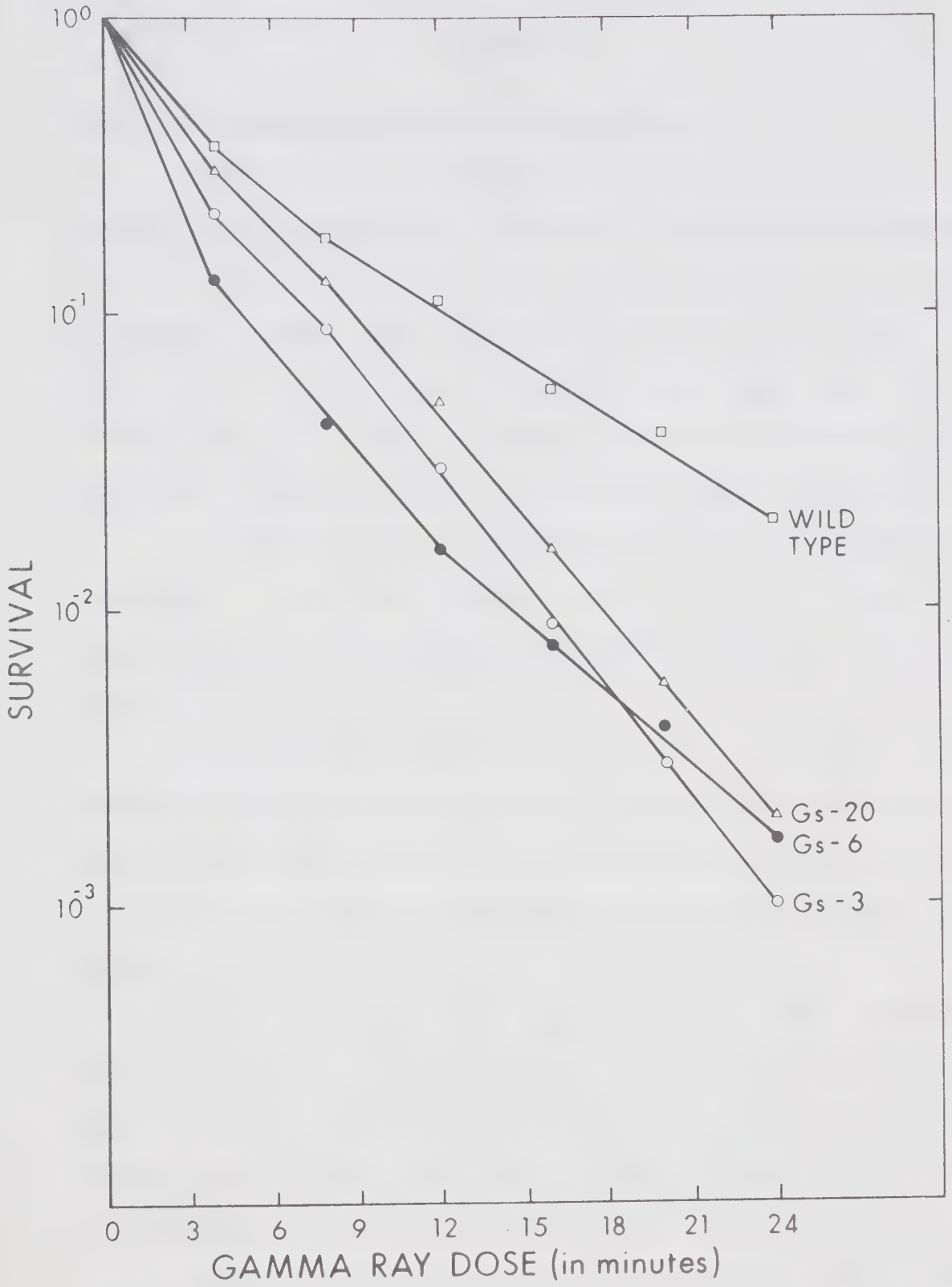

$10^2$
$10^1$
$10^0$
$10^{-1}$
PERCENT SURVIVAL
0
4
8
12
16
20
24
GAMMA RAY DOSE (in minutes)
WILD TYPE
(0) Gs-4
(0) Gs-2
(0) Gs-1

FIG. 2. Gamma-ray survival curves for a wild type strain, pe (Y8743m)a and three gamma-ray sensitive mutants, gs-3, gs-6 and gs-20 of Neurosopora crassa. The survival curves represent the average of three independent experiments. Dose rate: 6.7 Krads $min^{-1}$.


SURVIVAL
$10^0$
$10^{-1}$
$10^{-2}$
$10^{-3}$
WILD TYPE
Gs - 20
Gs - 6
Gs - 3
0
3
6
9
12
15
18
21
24
GAMMA RAY DOSE (in minutes)

# RESULTS

## Isolation and gamma-ray survival characteristics:

On the basis of the ability of a conidial cell to form a colony after irradiation with a given dose of gamma-rays, six gamma-ray sensitive mutants were isolated from the wild type strain, pe (Y8743m)a of Neurospora crassa. Ultraviolet-light was used as the mutagen. Gamma-ray-survival curves of mutants designated as gs-1, gs-2, gs-4, (Fig.1) and gs-3, gs-6, gs-20, (Fig. 2) together with the isogenic parent strain pe (Y8743m) a (hereafter referred to as wild type), were obtained.

The gamma-ray dose response curve of the wild type strain showed an inflexion at 26.8 Krads (yielding a survival of 37%). After 26.8 Krads, increasing dose attained a linear relationship with decreasing survival.

The gamma-ray-survival curves of mutants, gs-1, gs-3 and gs-20 showed throughout the dose-range used, a steeper slope than the dose response curves of the wild type. No obvious inflexion points, as observed in the wild type strain,were obtained from the survival curves of these mutants.

Like the wild type, mutant gs-6 appeared to show a gradual decline in the slope of its survival curve after a dose of 26.8 Krads. At dose levels which yielded in the wild type strain a survival <10%, the survival curve of mutant gs-6 showed a further decrease in slope thereby attaining the slope of the survival curve for the wild type.

Gamma-ray dose response curves of mutants gs-4 and gs-2 showed a slight decline in the slope after the dose of 26.8 Krads and then became almost parallel to the dose-response curve of mutant gs-1. However,

an inflexion was observed in the survival curve of mutant gs-4 at the dose of 80.4 Krads, decreasing the slope of the curve beyond this point. It should be noted that no survival was observed in mutant gs-2 and gs-4, beyond the dose of 107.2 Krads (which yielded a survival of 5.7% in the wild type strain).

A consistent decline in the dose reduction factors from higher (37%) to lower (3.7%) survival levels was observed in all the gamma-ray sensitive mutants (Table I).

TABLE I. DRF for gamma sensitivity at three survival levels of gamma-sensitive mutants of Neurospora crassa.

| Survival (%) | Gamma-ray sensitive mutants | | | | | |
|---|---|---|---|---|---|---|
| | gs-1 | gs-2 | gs-3 | gs-4 | gs-6 | gs-20 |
| 37 | 0.75 | 0.55 | 0.65 | 0.60 | 0.50 | 0.85 |
| 10 | 0.61 | 0.46 | 0.61 | 0.51 | 0.41 | 0.74 |
| 3.7 | 0.57 | 0.46 | 0.57 | 0.51 | 0.44 | 0.65 |

$$\text{Dose reduction factor (DRF)} = \frac{\text{dose for a given survival of a particular mutant}}{\text{dose for the same survival of the wild type}}$$

The above observation was taken to indicate that when compared with the wildtype strain, the degree of gamma-ray sensitivity of all the six mutants increased with increasing dose of gamma rays.

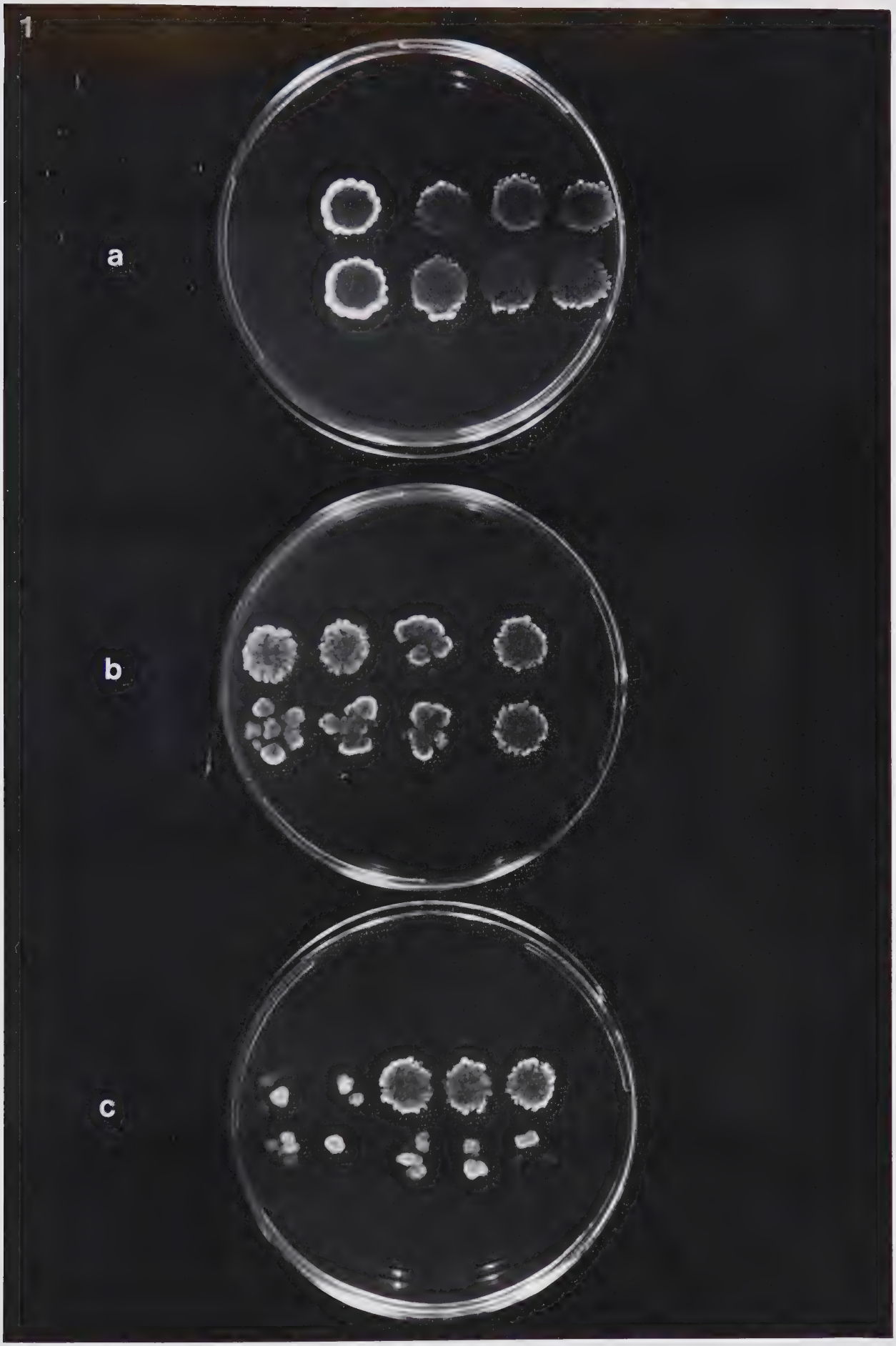
Mutant gs-6 showed the lowest DRF values at all the three levels of survival, being the most sensitive to gamma-rays followed by mutanst gs-2, gs-4, gs-3, gs-1 and gs-20 in the order of decreasing sensitivity.

Plate I. Photographs of plates showing the qualitative test for gamma-ray sensitivity. Each colony in the plates was grown from a drop of conidial suspension ($1x10^5$ conidia per ml). Plates were incubated at 25°C for three days.

a) Colonies grown from control (unirradiated) conidial suspensions of eight ascospore cultures of a tetrad originating from a cross: pe (Y8743m) a x 46-5, al-2, pan-2(B3), tryp-2, A.

b) the colonies of the same tetrad as in sub. a, but grown after irradiation of the conidial suspension of individual ascospore cultures with gamma-rays (dose: 134 Krads).

c) Colonies grown from gamma-irradiated (dose: 134 Krads) conidial suspensions of strains, 46-5, al-2, al-2, pan-2(B3), tryp-2, A; 467, pan-2(B5), ad-1, a; pe(Y8743m) a (colonies 3,4,5 respectively, upper row); six gamma-ray sensitive mutants, gs-1, gs-2, gs-3, gs-4, gs-6, and gs-20 and mutant uvs-3 (colonies 1 and 2 upper row, and 6,7,8,9 and 10 lower row, respectively). The uv-light and x-ray sensitive mutant uvs-3 was used as a reference strain for the gamma-ray sensitivity test.


1
a
b
c

The qualitative test for gamma-ray sensitivity of the mutants gs -1, gs-2, gs-3, gs-4, gs-6 and gs-20 compared with three wild type strains, pe (Y8743m)a; 46-5, al-2, pan-2(B3), tryp-2,A and 467 ad-1, pan-2(B5),a, is shown in P ate I. After receiving an equivalent dose of 134 Krads, the mutants showed no or very poor growth whereas the three wild type strains grew and produced mycelia.

TABLE II. Segregation of gamma ray sensitivity (gs) with respect to mating type (A/a) and auxotrophic markers: pan-2, tryp-2 and al-2, in unordered tetrads from various crosses

| Cross | Number analyzed | Tetrad types with respect to gamma-ray sensitivity and mt | | | pan-2 | | | tryp-2 | | | al-2 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PD | NPD | TT | PD | NPD | TT | PD | NPD | TT | PD | NPD | TT |
| 46-5,al-2,pan-2(B3), tryp-2,A x gs-1,a | 8 | 2 | 1 | 5 | 3 | 0 | 5 | 3 | 0 | 5 | 0 | 2 | 6 |
| 46-5,al-2,pan-2(B3), tryp-2,A x gs-2,a | 10 | 4 | 0 | 6 | 2 | 2 | 6 | 1 | 1 | 8 | 1 | 2 | 7 |
| 46-5,al-2,pan-2(B3), tryp-2,A x gs-3,a | 10 | 2 | 1 | 7 | 1 | 2 | 7 | 1 | 2 | 7 | 1 | 1 | 8 |
| 46-5,al-2,pan-2(B3), tryp-2,A x gs-4,a | 6 | 2 | 2 | 2 | 2 | 1 | 3 | 3 | 0 | 3 | 1 | 1 | 4 |
| 46-5,al-2,pan-2(B3), tryp-2,A x gs-6,a | 8 | 1 | 1 | 6 | 5 | 0 | 3 | 5 | 0 | 3 | 1 | 1 | 6 |
| 46-5,al-2,pan-2(B3), tryp-2,A x gs-6,a | 6 | 1 | 1 | 4 | 1 | 1 | 4 | 0 | 1 | 5 | 1 | 2 | 3 |

PD = Parental ditype; NPD = Nonparental ditype; TT = Tetra type

Plate II. Photographs of plates showing the segregation of gamma-ray sensitivity. Each colony in the plates was grown from a drop of conidial suspension ($1x10^5$ conidia per ml) irradiated with gamma-rays (dose: 134 Krads).

Plates were incubated at 25°C for three days.

a) Colonies of eight ascospore cultures of a tetrad originating from a cross: gs-1,a x 46-5, al-2, pan-2(B3), tryp-2, A.

b) Colonies of eight ascospore cultures of a tetrad originating from a cross: gs-2, a x 46-5, al-2, pan-2(B3), tryp-2, A.

c) Colonies of eight ascospore cultures of a tetrad originating from a cross: gs-3, a x 46-5, al-2, pan-2 (B3), tryp-2, A.

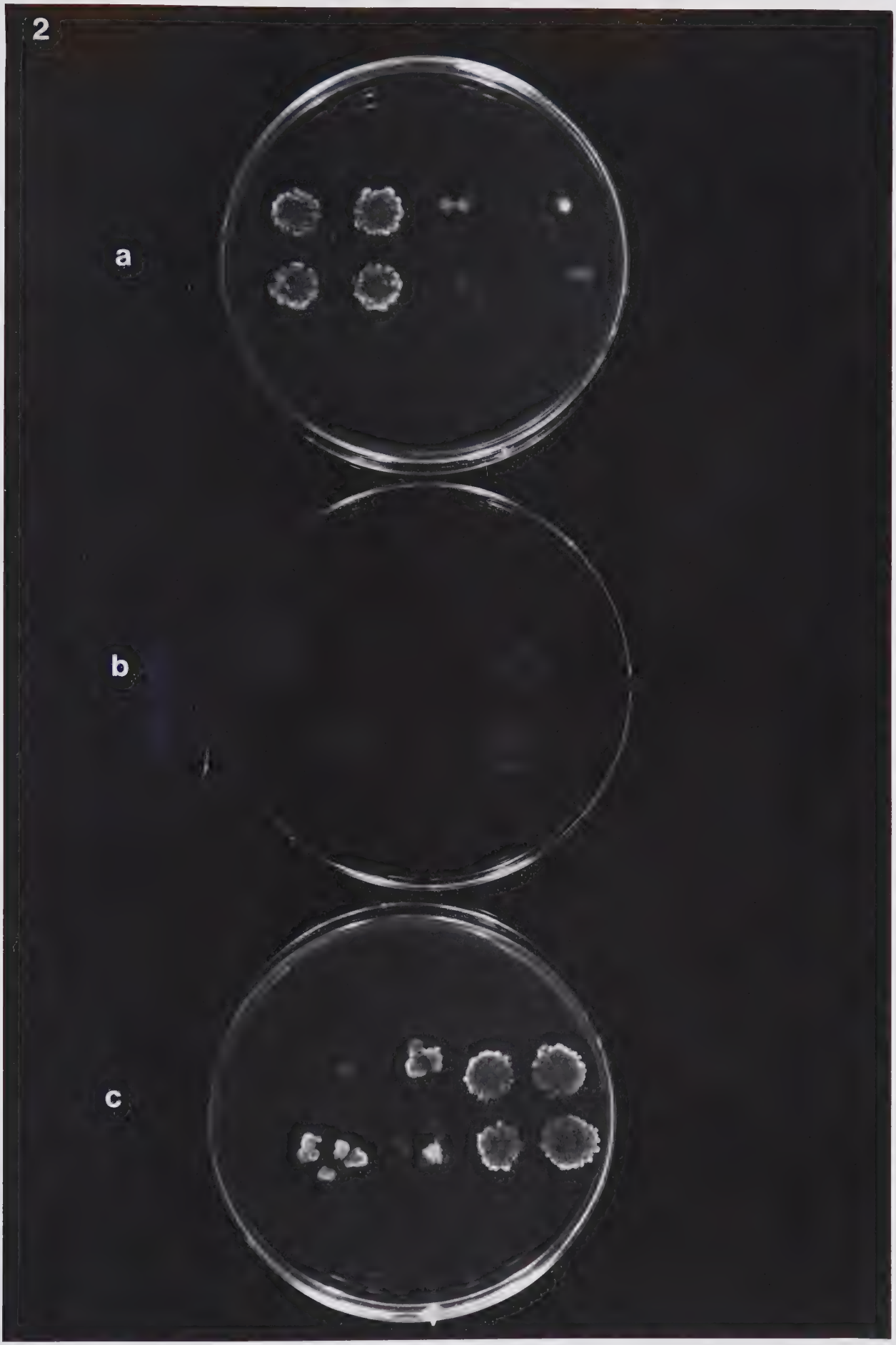
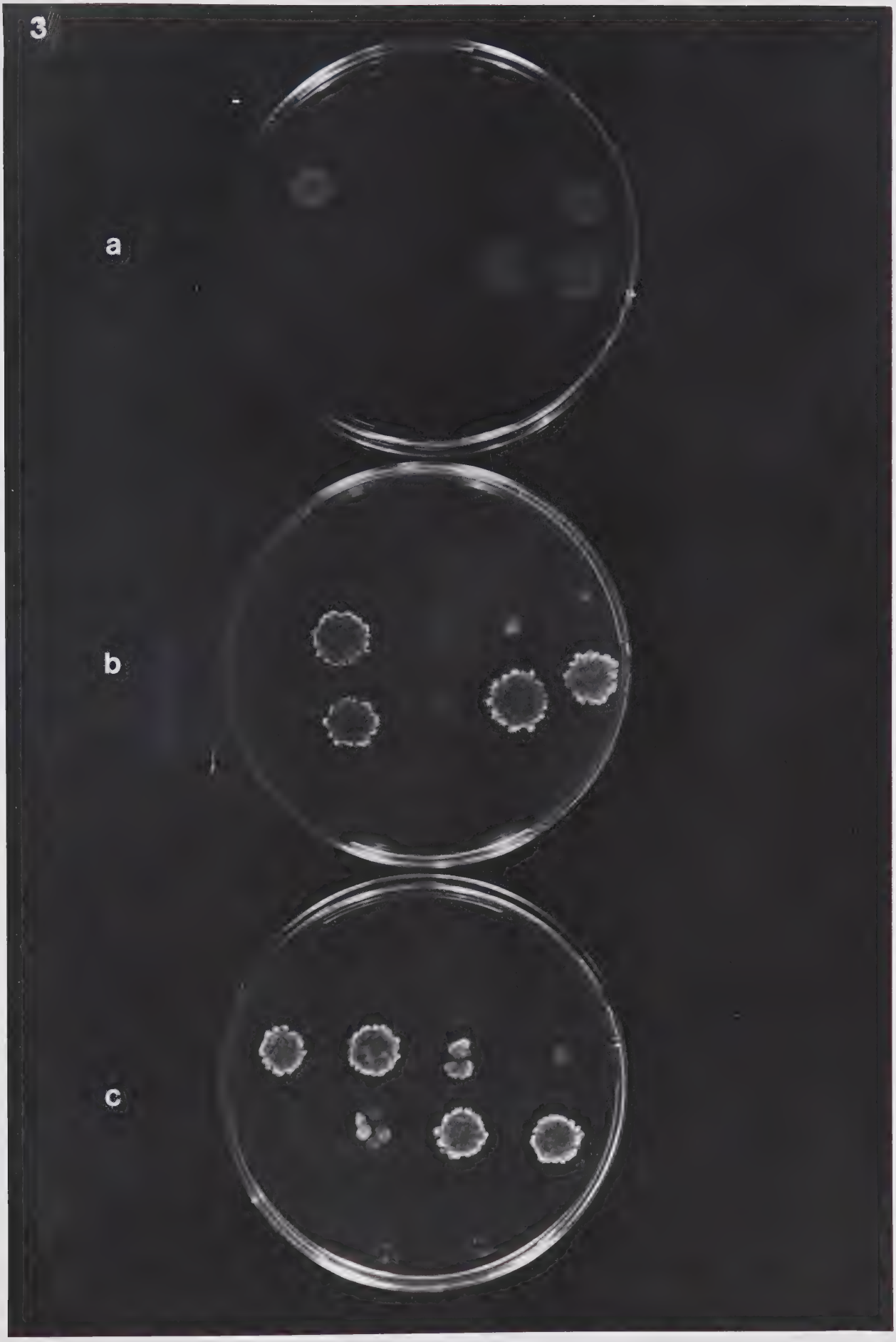

2
a
b
c

Plate III. Photographs of plates showing the segregation of gamma-ray-sensitivity. Each colony in the plates was grown from a drop of conidial suspension ($1x10^5$ conidia per ml) irradiated with gamma-rays (dose: 134 Krads). Plates were incubated at 25°C for three days.

a) Colonies of eight ascospore cultures of a tetrad originating from a cross: gs-4, a x 46-5, al-2, pan-2(B3), tryp-2, A.

b) Colonies of eight ascospore cultures of a tetrad originating from a cross: gs-6 a x 46-5, al-2, pan-2(B3), tryp-2 A.

c) Colonies of eight ascospore cultures of a tetrad originating from a cross: gs-20,a x 46-5, al-2, pan-2(B3), tryp-2, A.


3
a
b
c

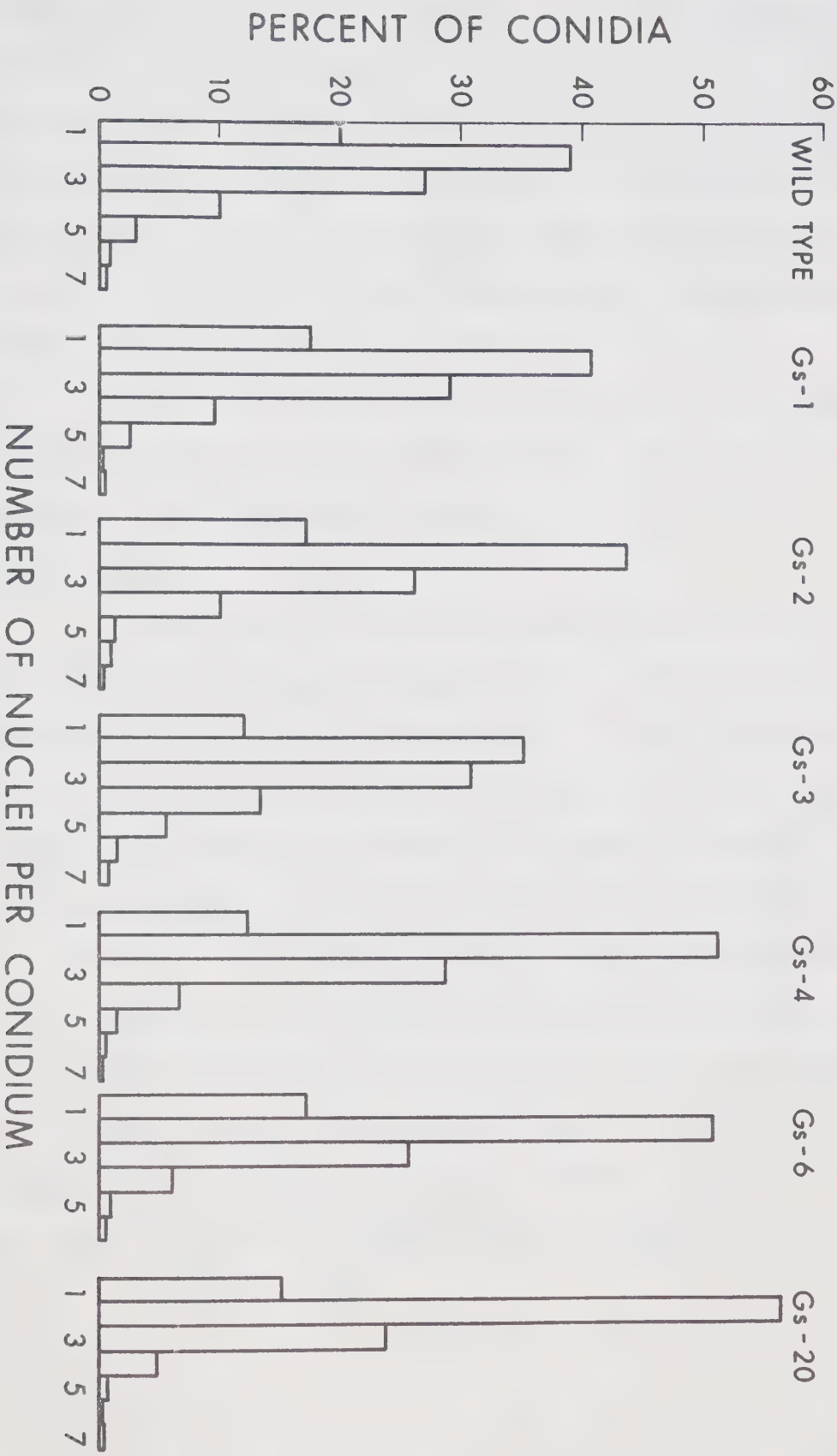
Genetic analysis:

To test for chromosomal inheritance of gamma-ray sensitivity, each of the mutants, gs-1, gs-2, gs-3, gs-4 and gs-20, was crossed with strain 46-5 al-2, pan-2(B3), tryp-2 A. A 4:4 segregation pattern was observed for gamma-ray sensitivity in all the crosses (Plates 2 and 3), indicating that gamma-ray sensitivity in each of the mutant strain was under the control of a single nuclear gene.

Unordered tetrads were analyzed from each cross and the results are presented in Table II. Because of the limited number of asci analyzed, it was not possible to draw a firm conclusion regarding the linkage relationship of the genes controlling radiation sensitivity in any of the mutant strain. However, out of 8 tetrads analyzed, no NPD'S (Non Parental Ditypes) were recovered with respect to pan-2 and tryp-2 markers (both on linkage group VI) in each of the cross, gs-1, a x 46-5 al-2, pan-2(B3), tryp-2A and gs-6, a x 46-5 al-2, pan-2(B5), tryp-2, A. Moreover, the ratio of TT (Tetratypes) is less than 2/3 in both cases. This type of tetrad distribution might indicate the possibility that genes controlling gamma-ray sensitivity in mutant gs-1 and gs-6 are located in linkage group VI close to the markers pan-2 or tryp-2 (see Esser and Kuenen, 1967).

Similarly, with respect to the marker mating type (A vs. a) no NPD'S were encountered in tetrads (10) originating from the cross gs-2 x 46-5 al-2 pan-2 (B3) tryp-2 A. This apparent lack of NPD'S together with a frequency of TT'S <2/3 is indicative for a possible linkage relationship between the gene gs-2 and the mating type locus A vs. a which is on linkage group I. The genes controlling gamma-ray sensitivity

FIG. 3. Distribution of nuclei in the conidia of wild type strain, pe (Y8743m) and six gamma-ray sensitive mutants of Neurospora crassa. The distributions are based on counts of 600-800 conidia. The average number of nuclei per conidium is 2.4, 2.4, 2.4, 2.7, 2.4, 2.2., 2.2 in wild type, gs-1, gs-2, gs-3, gs-4, gs-6, and gs-20 respectively.


PERCENT OF CONIDIA
0
10
20
30
40
50
60
WILD TYPE
Gs-1
Gs-2
Gs-3
Gs-4
Gs-6
Gs-20
1 3 5 7
NUMBER OF NUCLEI PER CONIDIUM

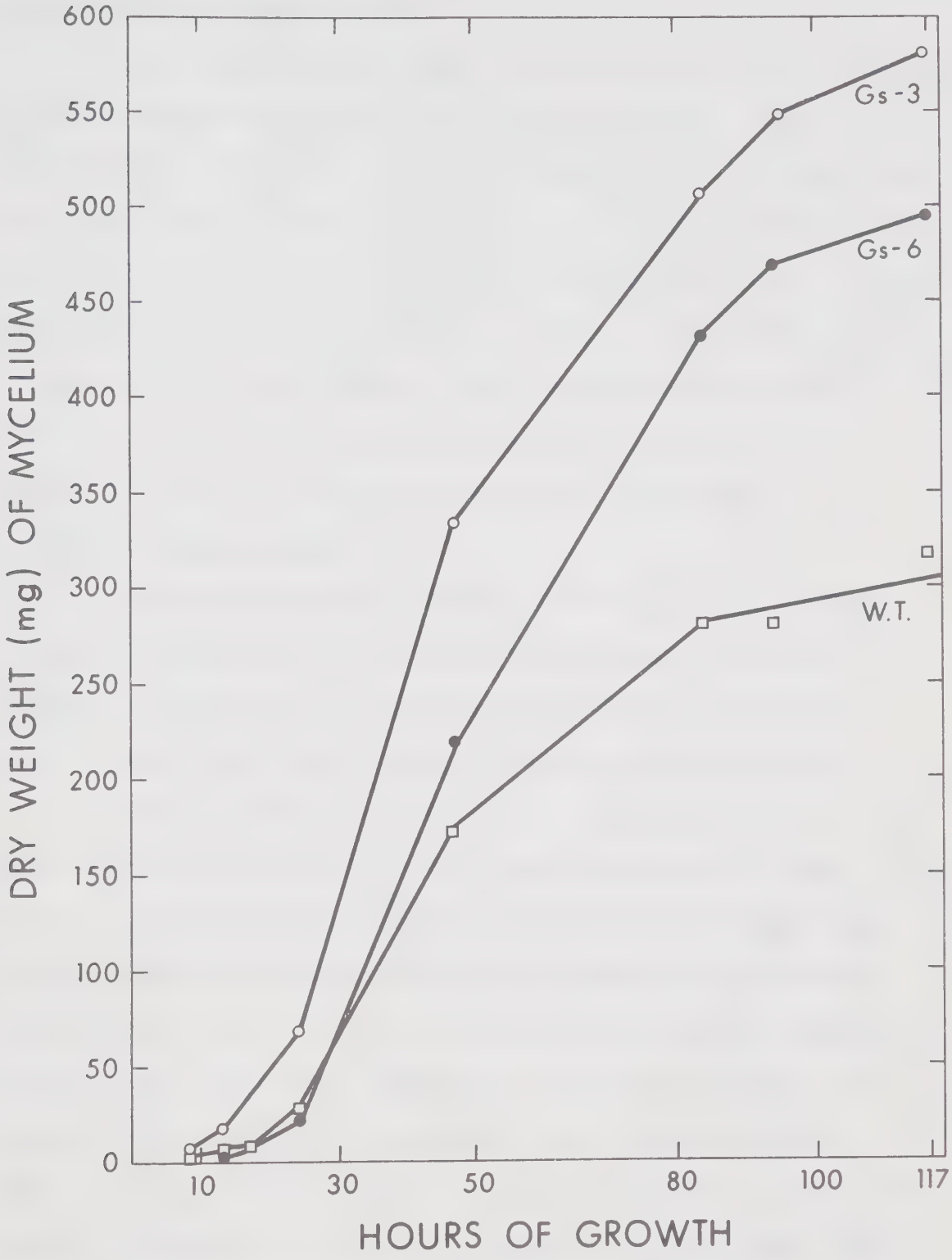
in mutant gs-3, gs-4 and gs-20 appeared to segregate independently from all the markers tested as revealed by the tetrad type pattern obtained from the crosses involving these mutants. In these crosses male sterility of gs-3 segregated from radiation sensitivity. The analysis of tetrad type patterns from the cross involving mutant gs-4 did not allow for any conclusion concerning the linkage relationship of gene gs-4 which controls gamma-ray sensitivity in that strain.

Attempts to perform complementation tests on the gamma-ray sensitive mutants were not successful because of the failure of heterokaryon formation between these mutant strains.

Nuclear numbers:

To establish the fact that the radiation sensitivity of mutants gs-1, gs-2, gs-3, gs-4, gs-6 and gs-20 was not due to a mutation affecting the number of nuclei per conidium, samples of conidia from sensitive mutants and wild type strain pe(Y8743m)a were stained and the nuclei were counted as described in Material and Methods. All gamma-ray sensitive mutants and wild type strains showed a comparable distribution of conidia with different number of nuclei (Fig. 3). The average number of nuclei per conidium was found to be 2.4 in mutants gs-1, gs-2, gs-4 and wild type pe(Y8743m)a. Mutants gs-6 and gs-20 possessed 2.2 nuclei/conidium, whereas mutant gs-3 had 2.7 nuclei/conidium. The low number of nuclei/conidium in gs-6 and gs-20 may partly be due to the poor staining of nuclei in these strains which made it difficult to count the correct number especially in conidia with a high number of nuclei.

FIG. 4. Growth curves of wild type strain, pe (Y8743m) and gamma-ray sensitive mutants, gs-3 and gs-6 of Neurospora crassa. The curves are based upon the average of two experiments.


600
550
500
450
400
350
300
250
200
150
100
50
0
DRY WEIGHT (mg) OF MYCELIUM
Gs-3
Gs-6
W.T.
10
30
50
80
100
117
HOURS OF GROWTH

Growth Studies in Liquid Medium.

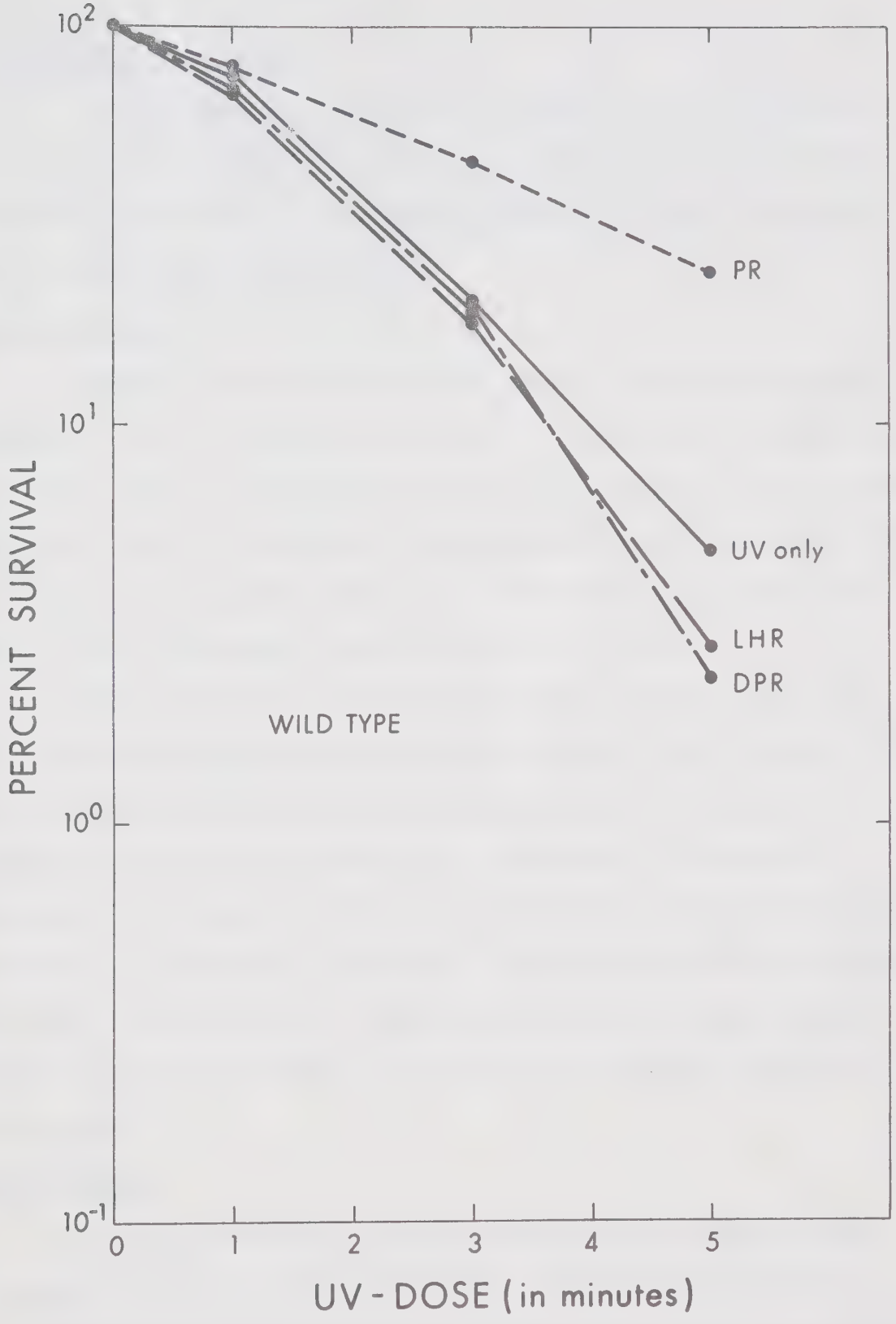
In growth tests mutant gs-6 showed very slow growth on slants of minimal medium as comapred to wild type pe (Y8743m)a. The other mutants however exhibited normal growth comparable to wild type except mutant gs-3 which was found to be fast growing on minimal medium slants. When mutants gs-6, gs-3 and wild type pe (Y8743m)a were grown on minimal liquid medium, both mutant gs-6 and gs-3 showed a faster growth rate as compared to wild type (Fig.4). These results were interpreted to indicate that the slow growth of the mutant gs-6 on minimal solid medium was not due to any inherent nutritional requirement.

uv-Survival Characteristics:

The uv-dose-response curves of mutants gs-1, gs-2 and gs-4 are shown in Figure 11 and those of mutants gs-3, gs-6 and gs-20 are presented in Figure 10. For comparison the survival curve of the isogenic parent strain pe (8743m)a is included in both the graphs.

Although the survival curves after uv-irradiation of all the mutant and the wild type strains were indistinguishable over most of the dose range tested, the dose response curves of wild type, gs-1, gs-3 and gs-6 showed characteristic shoulders at higher survival levels (≥80% survival of wild type). For mutant gs-1 the low dose shoulder is extended over a longer dose range when compared with wild type. The survival curve of mutant gs-4 appeared to have lost the shoulder characteristics at low uv-doses. Mutant gs-6 exhibited at higher dose levels (>1356 ergs $mm^{-2}$) increased uv-sensitivity when compared with the wild type strain (DRF = 0.73 at 2% survival level).

FIG. 5. Survival of conidia of wild type strain, pe (Y8743m) after uv-irradiation (dose rate: 11.3 ergs $mm^{-2}$ $sec^{-1}$) and post uv-irradiation treatments for PR(photoreactivation), LHR(liquid holding recovery) and DPR(delayed photoreactivation). The curves are based upon the average of three experiments.


$10^2$
$10^1$
$10^0$
$10^{-1}$
PERCENT SURVIVAL
PR
UV only
LHR
DPR
WILD TYPE
0
1
2
3
4
5
UV - DOSE (in minutes)

Post-irradiation Treatments:

Since mutant gs-6 was found to be sensitive to uv-irradiation as compared to its progenitor pe (8743m)a, attempts were made to further characterize this mutant by studying the effects of various treatments on the survival after uv-irradiation.

Photoreactivation:

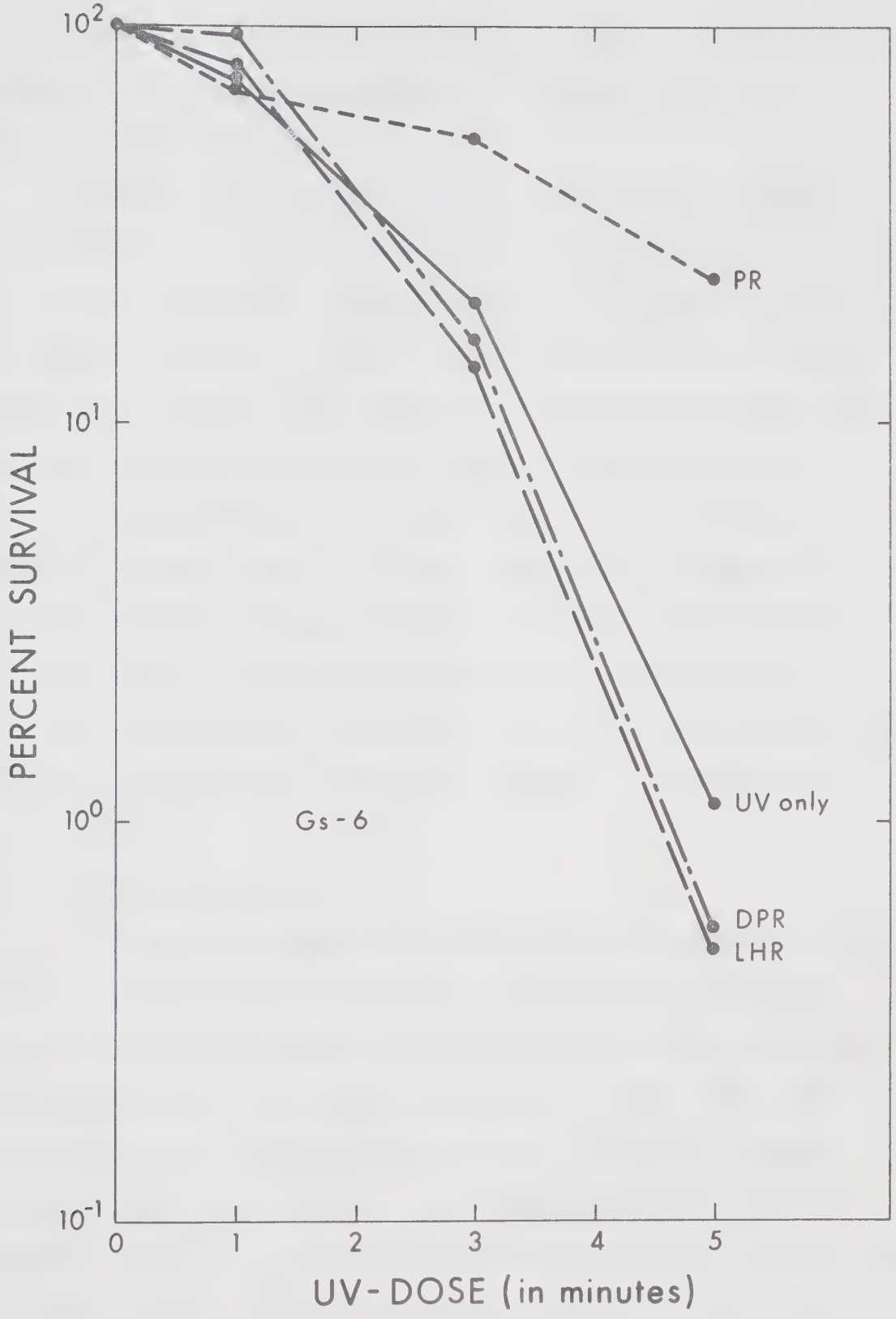
Exposure of wild type conidia to visible light for 30 minutes immediately after uv-irradiation gave rise to a significant increase in the survival (Fig. 5). The dose reduction factor amounted to 0.57 at the 37% survival level. The effect of photoreactivation was found to be more pronounced at lower survival levels. No photoreactivating effect was observed after holding the conidia in liquid for 6 hours.

The survival of conidia of mutant gs-6 was greatly increased after photoreactivation,with a dose reduction factor (0.54) close to the value obtained for wild type at the 37% survival level (Fig. 6). No photoreactivating effect was observed in this mutant at the dose of 678 ergs $mm^{-2}$ yielding a survival of about 70%. When the photoreactivation was delayed for 6 hours as in wild type, the response was lost at higher dose levels (>1356 ergs $mm^{-2}$). However, unlike in wild type,at a dose yielding 74% survival, delayed photoreactivation increased the survival in mutant gs-6.

Liquid Holding:

By holding conidia of the wild type strain in distilled water in the dark for 6 hours after uv-irradiation a slight but consistent loss of viability was observable when compared with the control (survival after immediate plating) as shown in Fig. 5. The effect of liquid holding

FIG. 6. Survival of conidia of gamma-ray sensitive mutant, gs-6 of Neurospora crassa after uv-irradiation (dose rate: 11.3 ergs $mm^{-1}$ $sec^{-1}$) and post uv-irradiation treatments for PR(photoreactivation), LHR(liquid holding recovery) and DPR(delayed photoreactivation). The curves are based upon the average of three experiments.


$10^2$
$10^1$
$10^0$
$10^{-1}$
PERCENT SURVIVAL
Gs-6
PR
UV only
DPR
LHR
0
1
2
3
4
5
UV-DOSE (in minutes)

is more conspicuous at lower survival levels (<20%) than at higher levels of survival. The dose reduction factor for liquid holding effect amounted to 0.88 at 5% survival.

Like wild type conidia, conidia of the mutant gs-6 showed a consistent decrease in survival upon holding in distilled water for six hours, the effect being more obvious at lower than at higher survival levels (Fig.6). However, at higher survival levels (>50%) the change in survival due to liquid holding appeared to be indistinguishable from the survival obtained after immediate plating. The dose reduction factor for liquid holding in mutant gs-6 was 0.90 at 5% survival and consequently similar to that of the wild type strain. Comparing the conidial survival of mutant gs-6, after liquid holding treatment with that of wild type, it appears that the effect of liquid holding is more severe in decreasing the viability in the former than in the latter, particularly at higher dose levels (2034 ergs $mm^{-2}$ - 3390 ergs $mm^{-2}$, Fig. 5 and Fig. 6).

Effect of Caffeine Treatment:

To investigate whether the treatment of caffeine has an influence on the repair systems operating in the irradiated Neurospora conidia of the wild type strain and of mutant gs-6, conidia were held in distilled water containing caffeine (0.25%) for 6 hours, after which they were plated (for liquid holding recovery) or exposed to photoreactivating light for 30 minutes (for delayed photoreactivation). The survival curves for conidia of the wild type strain and of mutant gs-6 after liquid holding treatment and delayed photoreactivation in the presence of caffeine are presented in Fig. 7 and Fig. 8 respectively.

FIG. 7. Survival of conidia of wild type strain, pe (Y8743m) of Neurospora crassa after uv-irradiation (dose rate: 11.3 ergs $mm^{-2}$ $sec^{-1}$) and post uv-irradiation treatments of LHR(liquid holding recovery), DPR(delayed photoreactivation) in caffeine-water solution (0.25%). The curves are based upon the average of three experiments.

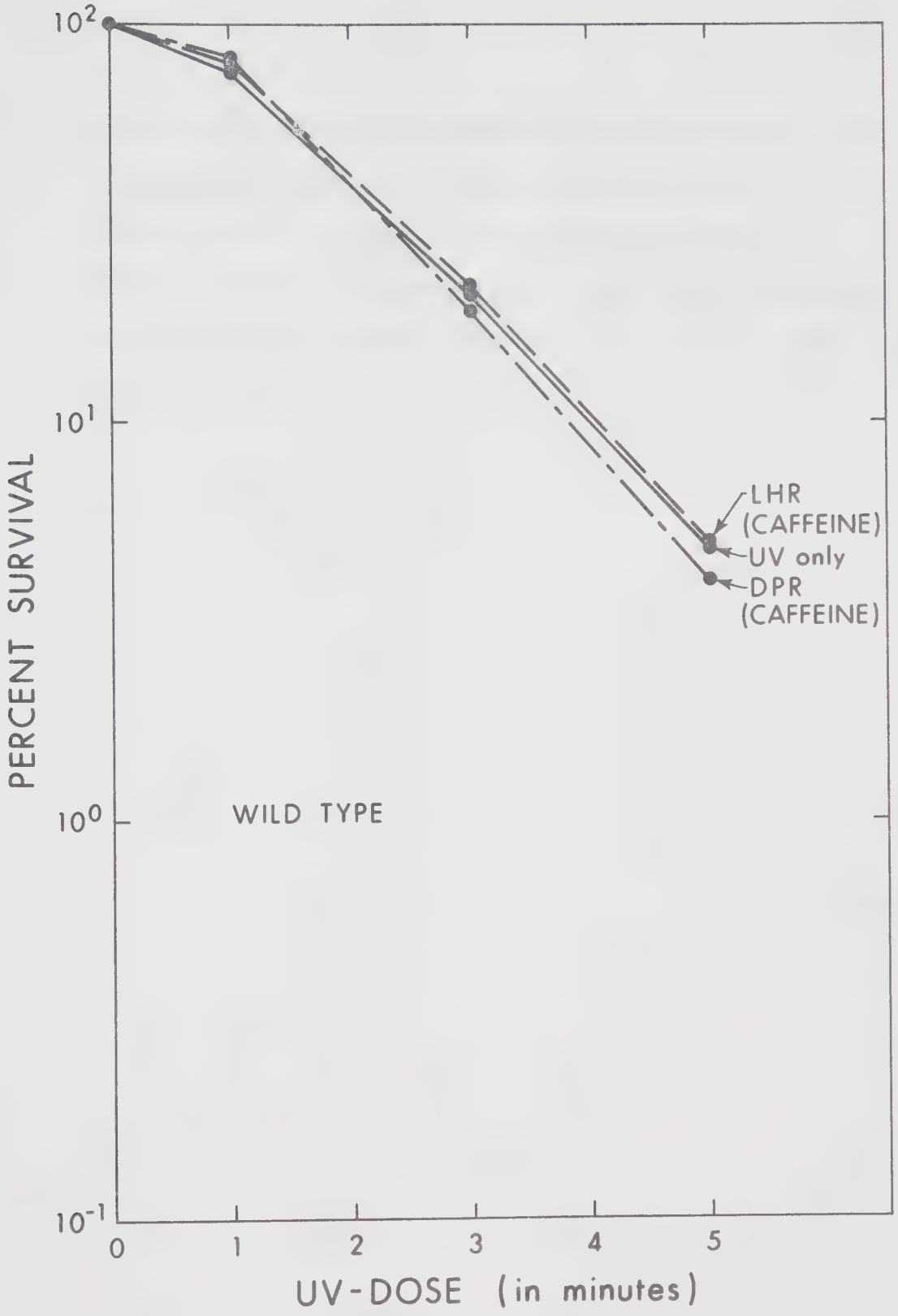
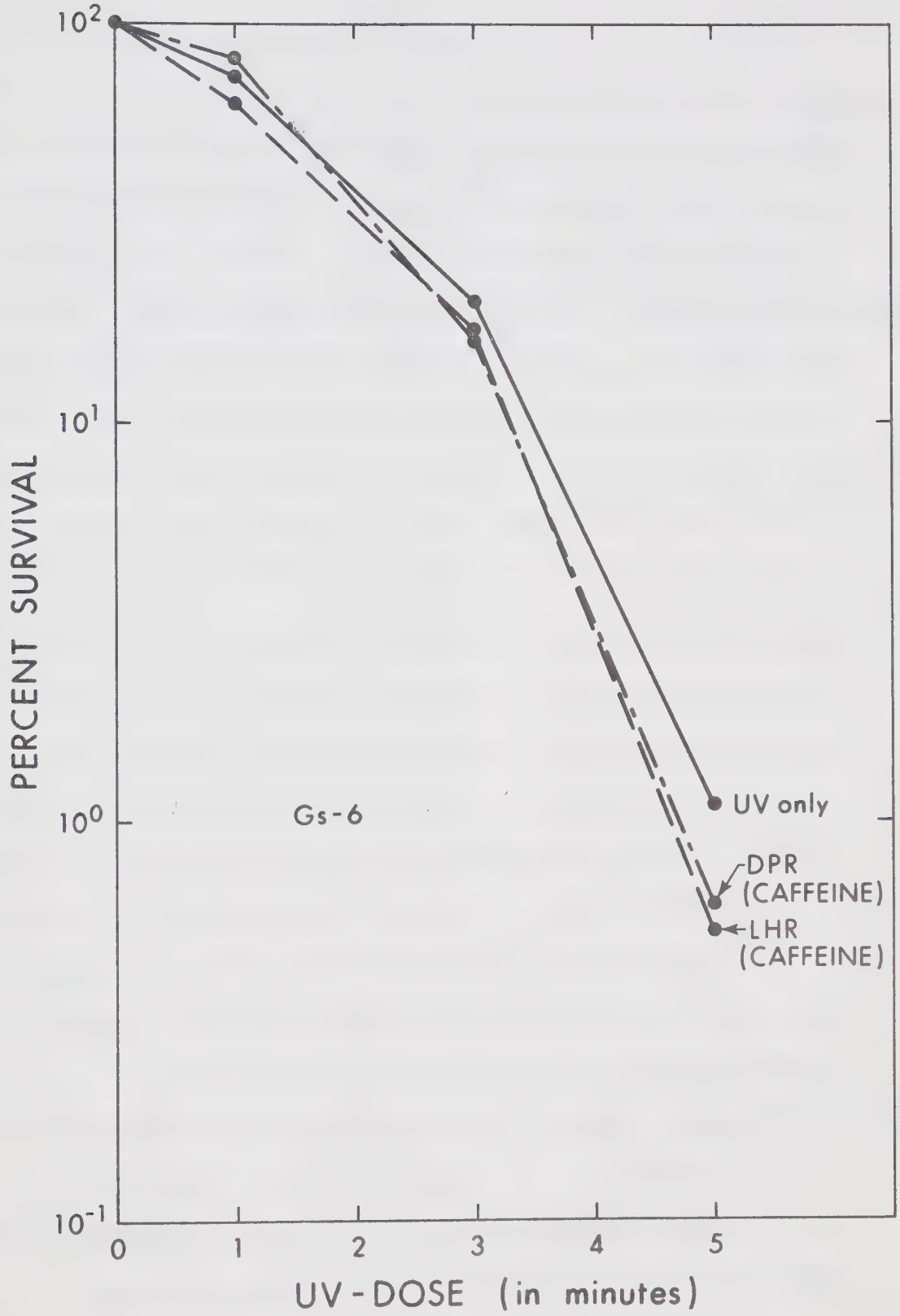

$10^2$
$10^1$
$10^0$
$10^{-1}$
PERCENT SURVIVAL
WILD TYPE
LHR (CAFFEINE)
UV only
DPR (CAFFEINE)
0
1
2
3
4
5
UV-DOSE (in minutes)

FIG. 8. Survival of conidia of the gamma-ray sensitive mutant, gs-6 of Neurospora crassa after uv-irradiation (dose rate: 11.3 ergs $mm^{-2}$ $sec^{-1}$) and post uv-irradiation treatments of LHR(liquid holding recovery) and DPR(delayed photoreactivation) in caffeine-water solution (0.25%). The curves are based upon the average of three experiments.


$10^2$
$10^1$
$10^0$
$10^{-1}$
PERCENT SURVIVAL
Gs-6
UV only
DPR
(CAFFEINE)
LHR
(CAFFEINE)
0
1
2
3
4
5
UV-DOSE (in minutes)

Comparison of survival curves in Figs. 5, 7 and Figs. 6, 8 showed that caffeine was ineffective in altering the mode of response in both, wild type and mutant gs-6 after the treatment of liquid-holding and delayed photreactivation. However, at the dose of 3390 ergs $mm^{-2}$ (i.e maximum dose used) the presence of caffeine in the suspension appeared to slightly elevate the survival levels of the wild type strain. No such effect was noticed for conidia of mutant gs-6 treated in a similar manner. The presence of caffeine in the suspending medium (distilled water) did not affect the survival of unirradiated conidia of both, the wild type strain and of mutant gs-6. The percent survival of wild type conidia after holding in distilled water for 6 hours at 25°C was 64.4 $\pm$ 4.0 and after holding in the solution of caffeine (0.25%) for the same period of time was the same and amounted to 62.0$\pm$ 2.0% (average of three experiments). The corresponding percent survivals of conidia of the mutant gs-6 after holding for 6 hours in absence or presence of caffeine in the medium were found to be 64.0$\pm$ 4.0 and 62.0$\pm$ 3.0 respectively. However no visible colonies were observed when conidia of the wild type strain and of the mutant gs-6 were plated on the surface of solidified sorbose minimal medium containing 0.25% caffeine. It appears therefore, that caffeine inhibits the growth of conidia when present in the plating medium. Conidia treated with caffeine prior to plating on to solidified medium, are not affected.

Effect of Caffeine Treatment on a Caffeine Resistant Strain:

Mutant caf-rI was isolated from caffeine resistant mutant colonies induced in strain gs-20. This mutant is characterized by normal growth when cultured on agar solidified medium containing 0.25% caffeine. The mutant caf-rI was crossed with wild type strain pe (Y8743m)A and a

FIG. 9. Survival of conidia of caffeine resistant mutant, caf-rI of Neurospora crassa after uv-irradiation (dose rate: 11.3 ergs /$mm^{-2}$/$sec^{-1}$) when plated on medium containing 0.25% caffeine (broken line) and on medium without caffeine (solid line). The curves are based upon the average of two experiments.

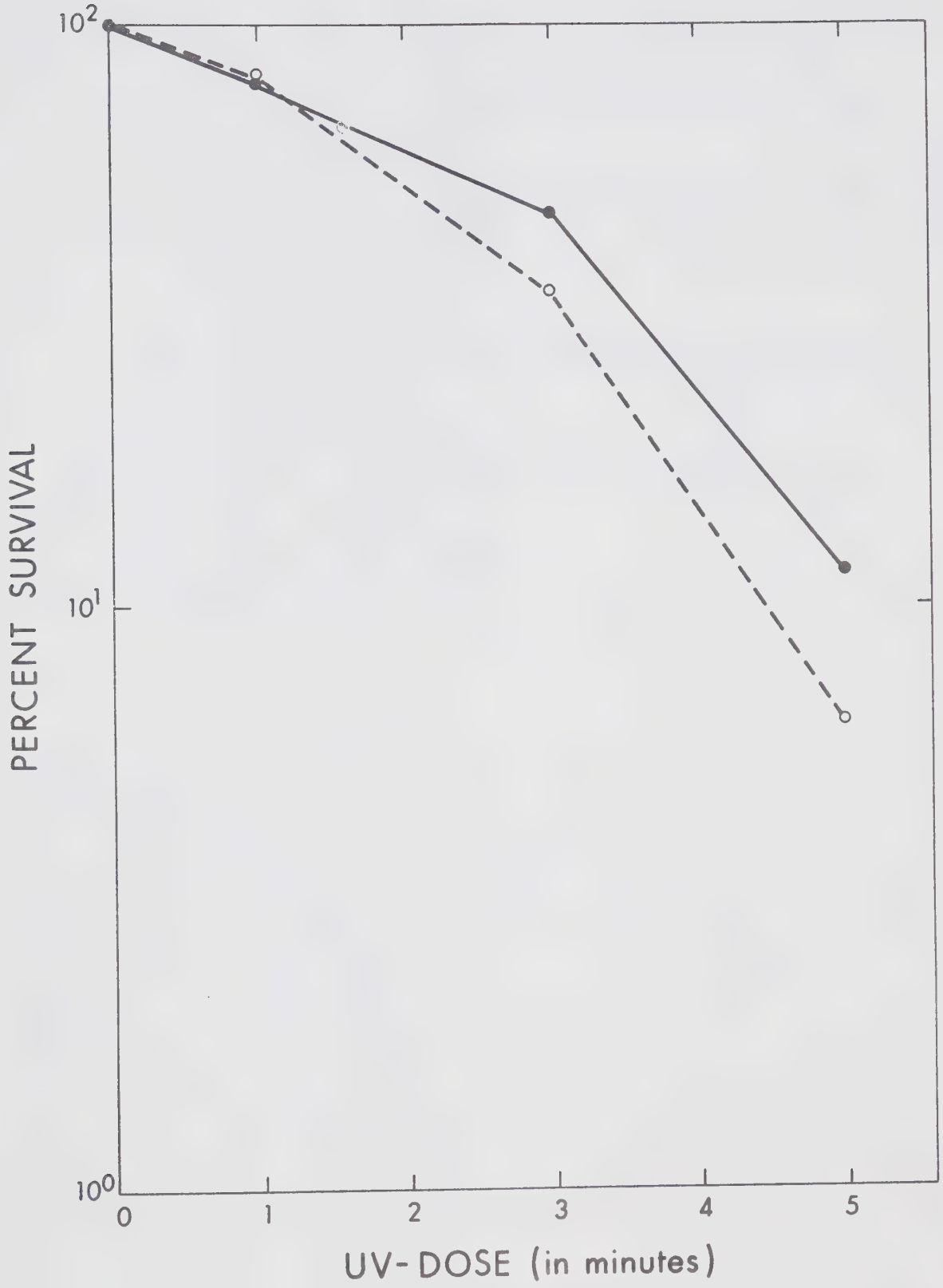

$10^2$
$10^1$
$10^0$
PERCENT SURVIVAL
0
1
2
3
4
5
UV-DOSE (in minutes)

Plate IV. Electron micrographs of mycelium of Neurospora crasa grown from unirradiated and irradiated (with gamma-rays) conidia of mutant gs-3.

1. Section of mycelium grown form unirradiated conidia
2. Section of mycelium grown from irradiated conidia, dose: 31 Krads.
3. Section of mycelium grown from irradiated conidia, dose: 93 Krads.
4. Section of mycelium grown from irradiated conidia, dose: 186 Krads.

Magnification: 68000 X

Abbrevations

W = cell wall

pm = plasma membrane

M = mitochondrium

ms = mesosome

V = vacuole

nu = nucleolus

N = nucleus

Gs-3

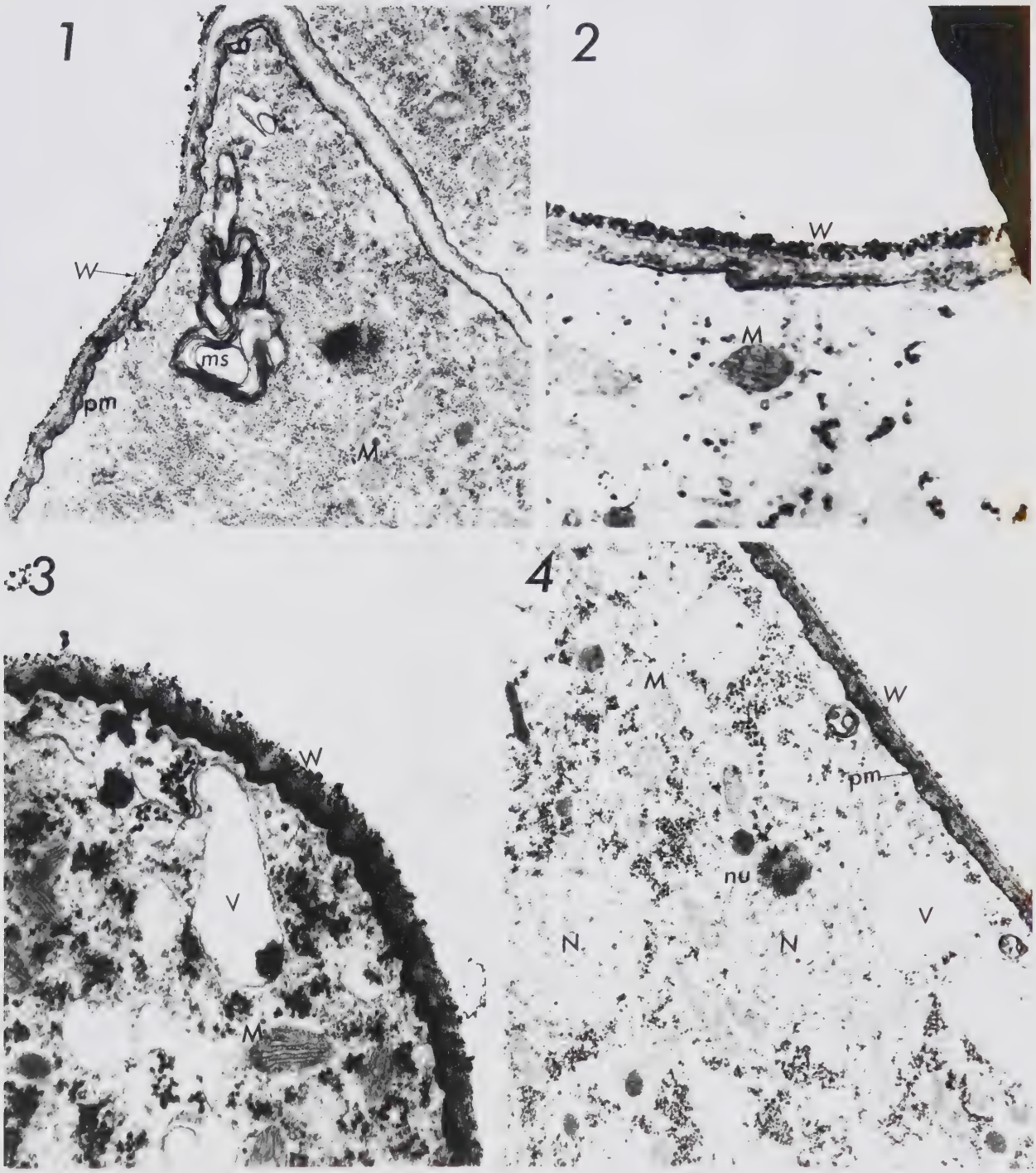


Plate V. Electron micrographs of mycelium of Neurospora crassa grown from unirradiated and irradiated (with gamma-rays) conidia of wild type strain, pe (Y8743m).

1. Section of mycelium grown from unirradiated conidia.
2. Section of mycelium grown from irradiated conidia dose: 31 Krads.
3. Section of mycelium grown from irradiated conidia, dose: 93 Krads.
4. Section of mycelium grown from irradiated conidia, dose: 186 Krads.

Magnification: 68000 X

Abbrevations:

W = cell wall

pm = plasma membrane

M = mitochondrium

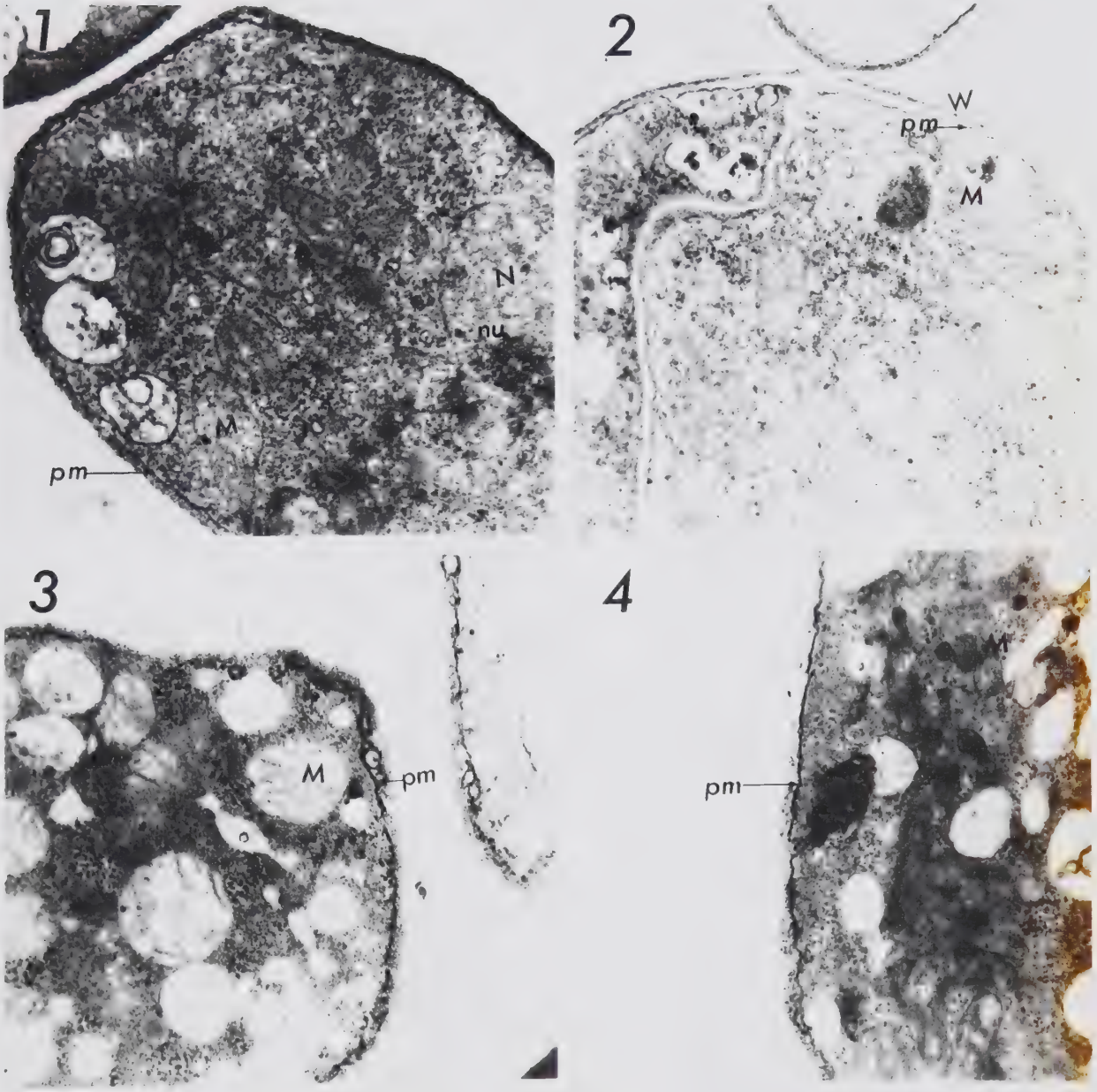
ms = mesosome

V = vacuole

nu = nucleolus

N = nucleus

W. T.



single ascospore culture resistant to caffeine and sensitive to gamma rays was isolated for further study. The use of this caffeine resistant mutant facilitate the study of the effect of caffeine present in the post-irradiation planting medium on conidial survival. Conidia of mutant caf-rI were irradiated with various doses of uv-light and subsequently, irradiated and control samples were plated on to two different media: one containing caffeine and the other without caffeine. The dose response curves of the mutant in the presence or the absence of caffeine are shown in Fig. 9. At survival levels in excess of 80% little difference in the survival was observed, whether conidia were plated onto medium containing caffeine or without caffeine. However at survival levels below 80%, the conidial survival decreased with increasing uv-dose. Regardless of the presence or absence of caffeine in the plating medium, the survival of unirradiated conidia amounted to 67.0 $\pm$ 9.0%.

Electron Microscopy:

The electron micrographs of mycelia grown from unirradiated and irradiated conidia of mutant gs-3 and wild type strain pe (Y8743m)a are shown in plate IV and plate V respectively. Examination of the electron micrographs revealed a characteristic effect of gamma-radiation on cell wall, mitochondria and vacuoles of both strains. The cell wall of mycelium of mutant gs-3 (plate IV, Fig. 1) shows three distinct layers, the outer-most consists of electron dense granules, the middle layer is distinguished by thinly distributed fibres in an electron transparent matrix and the innermost layer is densely packed with fibrils. The presence of fibrils and some electron dense granules embedded in the electron transparent matrix has also been observed in the cell wall of the wild type strain (plate V, Fig. 1). However, unlike mutant gs-3

the wild type strain does not exhibit distinct layers in the cell wall. No visible structural changes have been observed in the cell wall of mycelia grown from irradiated samples of conidia of both strains, mutant gs-3 and wild type strain (Plates IV and V; Figs. 2,3 and 4).

A conspicuous effect of gamma radiation consisted of swelling of the cell wall. This effect has been expressed as the thickness (in Å ) of the cell wall. The results are presented in Table III. The mean values shown in this table represents the average of wall thickness measurements of 4-6 different cells ( 4-6 determinations per cell). For the measurement of wall thickness only common but ultrastructurally different whwn compared with wild type, were chosen.

TABLE III. Cell wall thickness of unirradiated and irradiated conidia and mycelia produced from them in wild type ($gs^+$) and gamma sensitive mutant, (gs-3) of Neurospora crassa.

| Radiation-Dose (Krads) | Cell wall thickness in (Å) | | | |
|---|---|---|---|---|
| | Wild Type ($gs^+$) | | Mutant gs-3 | |
| | Conidia | Mycelia | conidia | Mycelia |
| control | 345.0 ± 42.3 | 323.0 ± 25.5 | 499.0 ± 31.5 | 605.0 ± 81.2 |
| 31 | 362.6 ± 57.0 | 391.7 ± 49.3 | 575.6 ± 84.5 | 1402.7 ± 221.1 |
| 93 | 457.8 ± 62.6 | 404.1 ± 53.0 | 629.2 ± 57.6 | 958.2 ± 102.6 |
| 186 | 418.0 ± 46.0 | 358.1 + 10.2 | 640.0 ± 45.5 | 675.4 ± 94.0 |

The cell wall of mycelia of mutant gs-3 is thicker (605Å) than the mycelial cell wall of the wild type (323Å) control (unirradiated) samples. This difference in wall thickness of both strains

is aggrevated in conidia treated with gamma-rays. An increase in the thickness to 1402 Å of the mycelial cell wall of gs-3, was observed when conidia producing the corresponding mycelia were irradiated with a dose of 31 Krads. Swelling of the mycelial cell wall of mutant gs-3 was also noticed when conidia were irradiated with higher doses of radiation e.g., 93 and 186 Krads. However, the swelling effect at these dose levels was less than the effect resulting from 30 Krads. The cell wall thickness of wild type mycelia after irradiation of conidia with equivalent doses of gamma rays did not substantially change and reached a maximum of 404 Å at 93 Krads.

Swelling of mitochondria due to irradiation manifested itself in larger intracristal spaces and was observed in both, mutant gs-3 and wild type strain. The effect was found to be more pronounced in mutant gs-3 when compared to wild type strain.

Many large and small vacuoles were observed in the sections of mycelia grown from conidia irradiated with various doses of gamma-rays. Some sections showed only a very limited amount of cytoplasm with an abundance of vacuoles in the remaining intracellular space. In the control (unirradiated) samples no or a very few vacuoles were observed in both strains (wild type and gs-3).

Electron micrographs of control and irradiated conidia of wild type and mutant gs-3 revealed essentially similar ultrastructure patterns as observed in the mycelia grown from them. The measurements of conidial cell wall thickness obtained from control and irradiated samples of both strains are recorded in Table III Swelling of the conidial cell wall appears to be more pronounced in mutant gs-3 when

FIG. 10. Forward mutation rate (ascending curves) to caffeine resistance and percent survival (descending curves) of wild type strain, pe (Y8743m) and gamma-ray sensitive mutants, gs-3, gs-6 and gs-20 of Neurospora crassa as a function of uv-dose (dose rate: 11.3 ergs $mm^{-2}$ $sec^{-1}$).

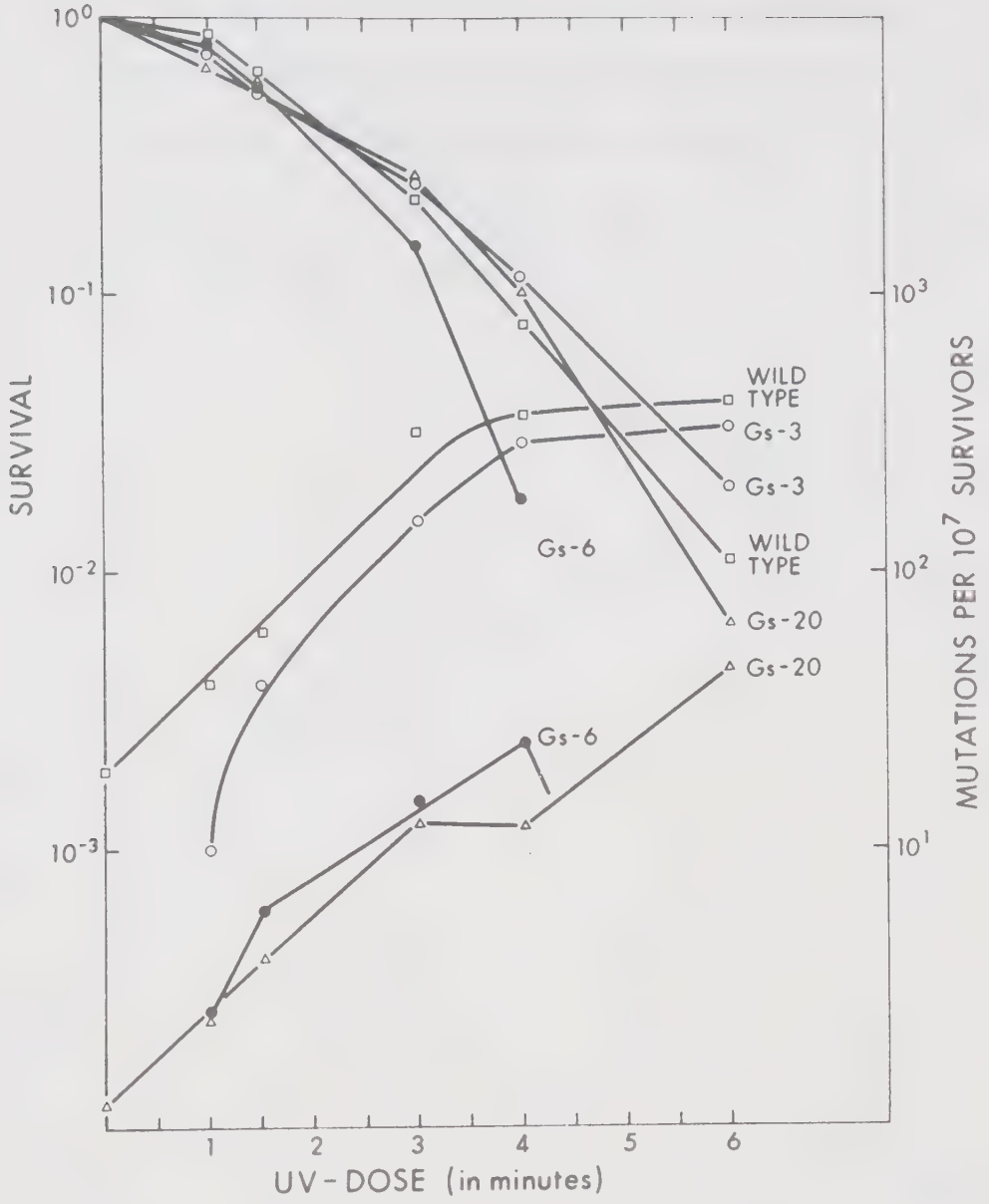
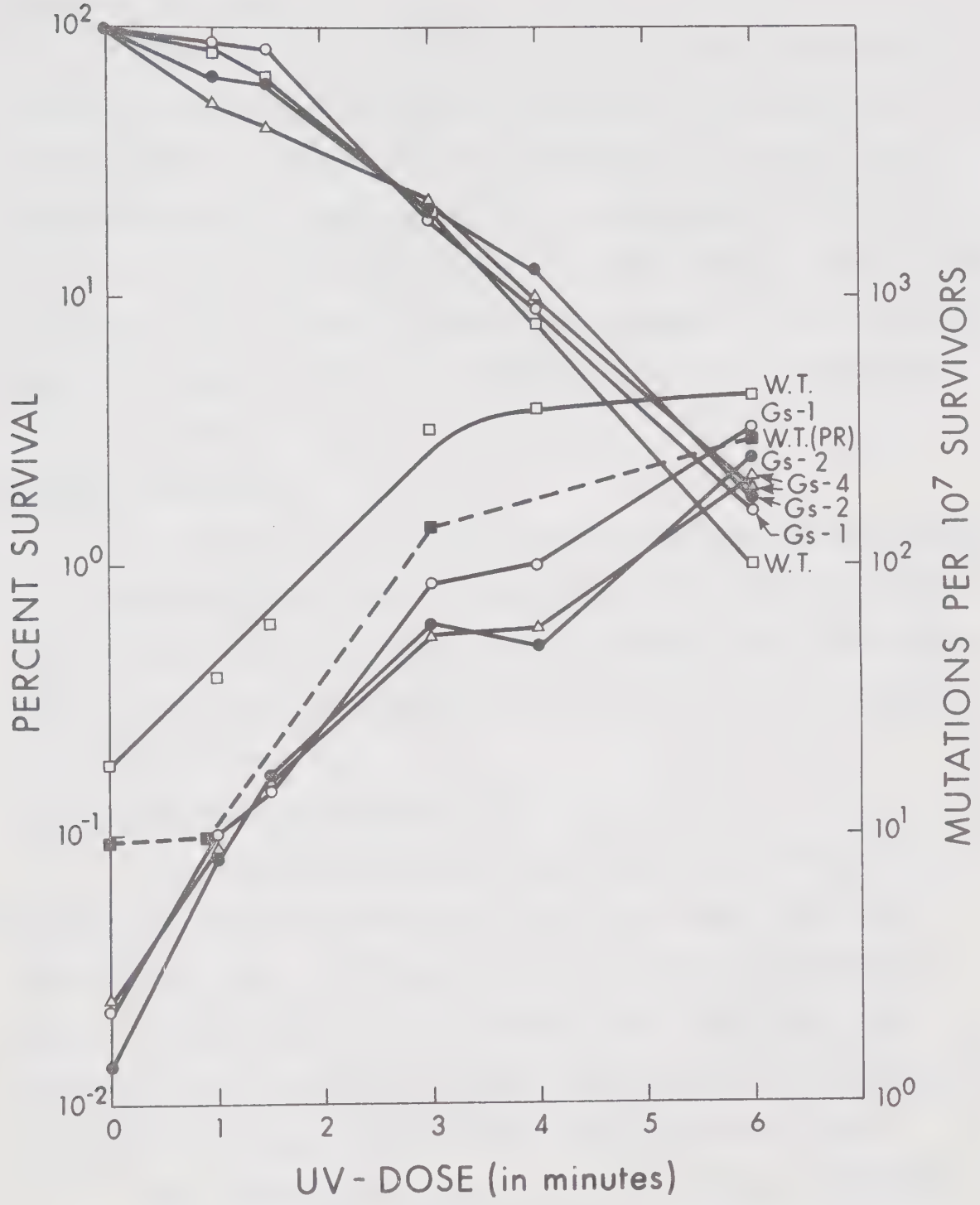

$10^0$
$10^{-1}$
$10^{-2}$
$10^{-3}$
SURVIVAL
$10^3$
$10^2$
$10^1$
MUTATIONS PER $10^7$ SURVIVORS
WILD TYPE
Gs-3
Gs-3
WILD TYPE
Gs-6
Gs-20
Gs-20
Gs-6
1
2
3
4
5
6
UV-DOSE (in minutes)

FIG. 11. Forward mutation rate (ascending curves) to caffeine resistance and percent survival (descending curves) of wild type strain, pe (Y8743m) and gamma-ray sensitive mutants, gs-1, gs-2, gs-4 of Neurospora crassa as a function of uv-dose (dose rate: 11.3 ergs $mm^{-2}$ $sec^{-1}$).


PERCENT SURVIVAL
MUTATIONS PER $10^7$ SURVIVORS
UV - DOSE (in minutes)
$10^2$
$10^1$
$10^0$
$10^{-1}$
$10^{-2}$
$10^3$
$10^2$
$10^1$
$10^0$
0
1
2
3
4
5
6
W.T.
Gs-1
W.T.(PR)
Gs-2
Gs-4
Gs-2
Gs-1
W.T.

compared to wild type.

Swelling effects on the cell wall due to gamma-irradiation treatment was found to be pronounced in mutant gs-3 after germination of the irradiated conidia. The wild type strain, on the other hand, reacted differently. Comparing the cell wall thickness of irradiated conidia with that of the mycelia grown from these conidia, no significant difference could be observed. These results suggest that the metabolic responses to gamma-irradiation are different in the two strains, gs-3 and the wild type.

uv-induced Mutability:

To study the effect of mutations causing gamma-ray-sensitivity on uv-induced mutability, forward mutations for caffeine or acriflavine resistance and back mutations for adenine prototrophy ($ad^+$) were induced with uv-light in appropriate gamma-ray-resistant (wild type) and gamma-ray-sensitive strains.

(i) Caffeine resistant mutants:

Mutations causing caffeine resistance were induced in all the six gamma-ray-sensitive mutants, gs-1, gs-2, gs-3, gs-4, gs-6, gs-20 and wild type strain pe (Y8743m)a. These data have been recorded in Table IV. Dose response curves of strains, gs-3, gs-6 gs-20 for uv-induced forward mutations to caffeine resistance are shown in Fig. 10 and those of strains gs-1, gs-2, and gs-4 are presented in Fig. 11.

The uv-induced mutations for caffeine resistance accumulated exponentially and reached a plateau at a dose yielding approximately 13% survival in wild type pe (Y8743m)a. The uv-induced caffeine resistant mutation curve for gs-3 closely followed the curve for the wild type

strain. However, on comparison with the wild type strain, a consistent reduction in the number of forward mutations induced by each uv-dose employed was observed in this mutant strain gs-3. The reduction in the number of caffeine resistant mutants in gs-3 is significant at the 5% probability level at lower uv-dose range ($\leq$2034 ergs $mm^{-2}$).

In mutant gs-6, the incidence of caffeine resistant mutations increased steadily until the point at which the wild type strain reached a plateau. After this point the mutation curve of gs-6 appeared to exhibit a sharp decline as no foward mutation was recovered in this stain at a uv-dose of 4060 ergs $mm^{-2}$.

In mutant gs-20, caffeine resistant mutations accumulated exponentially at approximately the same rate as in the irradiated wild type strain up to a dose of 2034 ergs $mm^{-2}$. After this point a plateau was established in gs-20(i.e., well before the wild type strain reached its plateau). Unlike that of the wild type strain,the plateau for mutant gs-20 was immediately followed by an exponential increase in mutation yield at a dose of 2712 ergs $mm^{-2}$ which yielded a survival of about 10%.

As compared to the wild type strain, a significant reduction in the yield of caffeine resistant mutations was observed in both mutant strains, gs-6 and gs-20, over the entire uv-dose used (at the 5% level of significance).

Qualitatively, a similar response was displayed by mutant gs-1, gs-2, and gs-4, for uv-induced mutations to caffeine resistance. Mutation yield increased exponentially in the three mutant strains up to a dose of 2034 ergs $mm^{-2}$. This initial increase, after reaching a

FIG. 12. uv-induced forward mutation rate to caffeine resistance of wild type strain, pe (Y8743m) and of gamma-ray sensitive mutants, gs-3, gs-6 and gs-20 of Neurospora crassa as a function of percent survival.

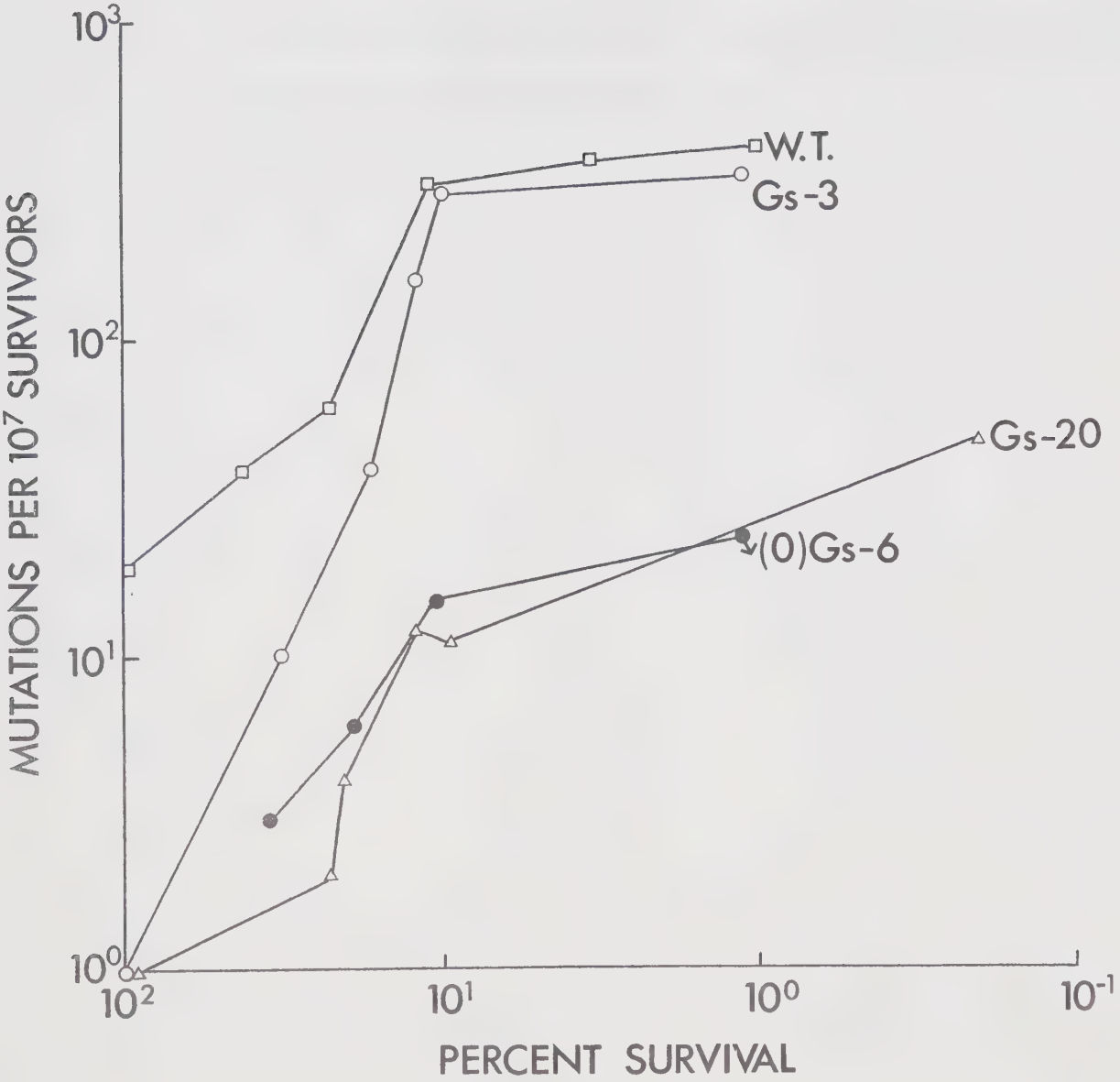

MUTATIONS PER $10^7$ SURVIVORS
$10^3$
$10^2$
$10^1$
$10^0$
W.T.
Gs-3
Gs-20
(0)Gs-6
$10^2$
$10^1$
$10^0$
$10^{-1}$
PERCENT SURVIVAL

FIG. 13. uv-induced forward mutation rate to caffeine resistance of a wild type strain, pe (Y8743m) and of three gamma-ray sensitive mutants, gs-1, gs-2, and gs-4 of Neurospora crassa as a function of percent survival.

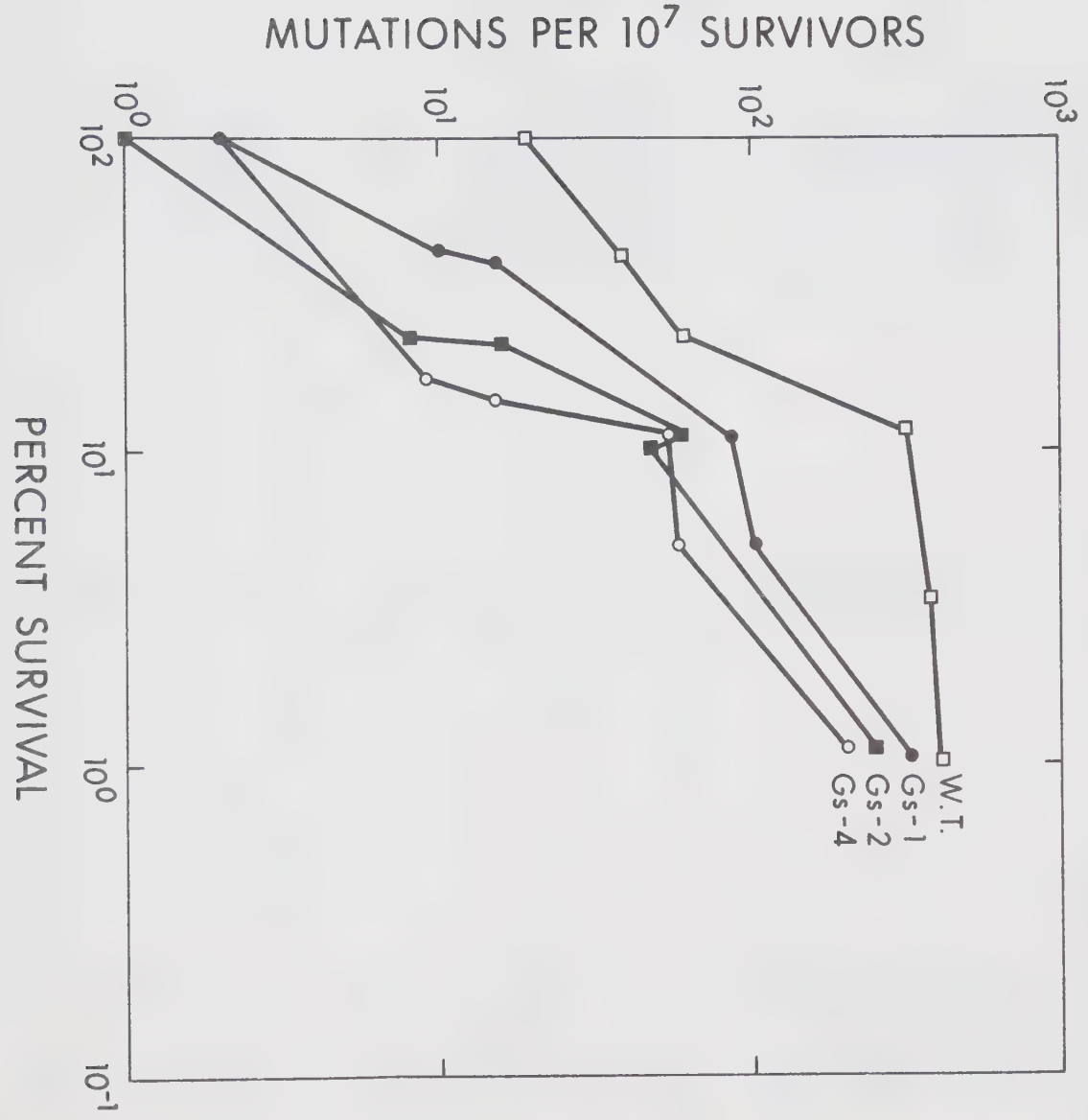

MUTATIONS PER $10^7$ SURVIVORS
$10^0$
$10^1$
$10^2$
$10^3$
PERCENT SURVIVAL
$10^2$
$10^1$
$10^0$
$10^{-1}$
W.T.
Gs-1
Gs-2
Gs-4

TABLE IV Frequency of spontaneous and uv-induced mutations for caffeine or acriflavine resistance in the wild type and gamma-ray-sensitive mutants of *N. crassa*

| Strain | uv-dose minutes | survival (%) | *Frequency of caffeine resistant mutations per $10^7$ survivors. | | *Acriflavine resistant mutants/ $10^7$ survivors |
|---|---|---|---|---|---|
| | | | Observed | Expected with 95% probability | |
| Wild type pe (Y8743m)a | 0 | 100.0 ± 5.6 | 19 | 14 - 25 | 0.42 |
| | 1 | 86.4 ± 4.3 | 39 | 32 - 48 | 0.38 |
| | 1.5 | 65.5 ± 7.1 | 60 | 51 - 70 | 0 |
| | 3 | 21.5 ± 1.7 | 316 | 269 - 371 | 0 |
| | 4 | 7.9 ± 0.4 | 366 | 293 - 452 | 0 |
| | 6 | 1.1 ± 0.1 | 407 | 286 - 561 | 0 |
| gs-1 pe a | 0 | 100.0 ± 6.2 | 2 | 1 - 4 | |
| | 1 | 86.7 ± 4.2 | 10 | 8 - 13 | |
| | 1.5 | 84.8 ± 3.4 | 15 | 12 - 18 | |
| | 3 | 19.5 ± 0.9 | 86 | 73 - 101 | |
| | 4 | 9.1 ± 0.6 | 101 | 83 - 121 | |
| | 6 | 1.6 ± 0.2 | 319 | 250 - 401 | |
| gs-2 pe a | 0 | 100.0 ± 5.0 | 1 | 1 - 3 | |
| | 1 | 66.2 ± 9.6 | 8 | 6 - 12 | |
| | 1.5 | 64.2 ± 4.9 | 16 | 12 - 20 | |
| | 3 | 22.4 ± 1.4 | 60 | 48 - 73 | |
| | 4 | 12.3 ± 0.7 | 47 | 36 - 59 | |
| | 6 | 1.8 ± 0.3 | 252 | 189 - 329 | |

TABLE IV Cont'd

| | | | | | |
|---|---|---|---|---|---|
| gs-3 pe a | 0 | 100.0 ± 11.8 | 1 | 0 - 2 | 0 |
| | 1 | 78.7 ± 9.30 | 10 | 7 - 14 | 0.31 |
| | 1.5 | 52.6 ± 4.58 | 39 | 30 - 50 | 1.93 |
| | 3 | 27.2 ± 2.3 | 157 | 128 - 189 | 4.70 |
| | 4 | 11.6 ± 2.1 | 294 | 238 - 359 | 0 |
| | 6 | 2.1 + 0.4 | 330 | 223 - 473 | 0 |
| gs-4 pe a | 0 | 100.0 ± 6.3 | 2 | 1 - 4 | |
| | 1 | 51.7 ± 4.3 | 9 | 6 - 13 | |
| | 1.5 | 43.0 ± 1.2 | 15 | 11 - 20 | |
| | 3 | 22.2 ± 2.6 | 55 | 45 - 66 | |
| | 4 | 9.1 ± 0.8 | 57 | 44 - 72 | |
| | 6 | 1.9 ± 0.2 | 199 | 150 - 259 | |

TABLE IV Cont'd

| | | | | | |
|---|---|---|---|---|---|
| gs-6 pe a | 0 | 100.0 ± 2.0 | 0 | 0 - 5 | 1.10 |
| | 1 | 80.7 ± 10.6 | 3 | 1 - 6 | 4.70 |
| | 1.5 | 56.7 ± 2.3 | 6 | 3 - 10 | 0.37 |
| | 3 | 14.6 ± 3.3 | 15 | 6 - 22 | 0 |
| | 4 | 1.8 ± 0.1 | 24 | 13 - 41 | 0 |
| | 6 | 0.0 ± 0.0 | 0 | 0 - 0 | 0 |
| gs-20 pe a | 0 | 100.0 ± 6.9 | 1 | 0.3 - 3 | 0 |
| | 1 | 65.8 ± 11.5 | 2 | 1 - 5 | 0 |
| | 1.5 | 62.8 ± 6.1 | 4 | 2 - 7 | 0 |
| | 3 | 29.1 ± 3.6 | 12 | 7 - 21 | 0 |
| | 4 | 10.0 ± 1.4 | 11 | 3 - 25 | 0 |
| | 6 | 0.6 ± 0.1 | 47 | 24 - 85 | 0 |

*The number of mutants produced at each dose represents the total number of induced spontaneous mutations for caffeine or for acriflavine resistance.

plateau was followed (at a dose of 2712 ergs $mm^{-2}$) by a further exponential increase in mutation rate. The mutation curves of these three mutants gs-1, gs-2 and gs-4 were found to be qualitatively similar to the mutation curve of gs-20. Mutants gs-1, gs-2 and gs-4 produced caffeine resistant mutants at a significantly reduced rate when compared to the wild type strain (at 5% level of significance). However for mutants gs-1 and gs-2 the reduction in mutation yield was not statistically significant at an uv-dose of 4060 ergs $mm^{-2}$.

When the frequencies for uv-induced caffeine resistance mutations of all the six gamma sensitive mutants are compared, the following order of decreasing mutational response can be established: gs-3>gs-1>gs-2, gs-4>gs-20>gs-6 (Table V).

Furthermore, all the six gamma-ray sensitive mutants showed a significant reduction in the frequency of spontaneous caffeine resistant mutation when compared with the wild type (Table IV) spontaneous mutation frequency for caffeine resistance. It is to be pointed out that when the frequency of spontaneous caffeine resistant mutations is substracted from the total yield of mutations obtained at each uv-dose level, the relative response of wild type and six gamma-ray sensitive mutants remain almost unchanged.

In Fig. 12 and Fig. 13 the yields of caffeine resistant mutants is plotted as a function of survival. It is evident that the wild type strain yielded more caffeine resistant mutants at all levels of survival when compared to the gamma-ray sensitive strains.

The wild type strain showed a steady increase in mutation yield at higher survival levels followed by a sharp increase at

TABLE V. Total frequency of spontaneous and uv-induced caffeine resistant mutants in the wild type and gamma-ray-sensitive mutants of Neurospora crassa.

| Strain | Total number of Survivors $\times 10^6$ | *Total number of mutant colonies | * Frequency of caffeine resistant mutants per $10^7$ survivors. | |
|---|---|---|---|---|
| | | | Observed | Expected with 95% Probability |
| gs$^+$,pe(Y8743m) | 171.4 | 991 | 58 | 54 - 62 |
| gs-1 | 234.3 | 514 | 22 | 20 - 24 |
| gs-2 | 167.8 | 320 | 19 | 17 - 21 |
| gs-3 | 126.4 | 330 | 26 | 23 - 29 |
| gs-4 | 175.6 | 335 | 19 | 17 - 21 |
| gs-6 | 169.9 | 45 | 3 | 2 - 4 |
| gs-20 | 121.3 | 54 | 4 | 3 - 6 |
| ** gs$^+$-pe(Y8743m) | 66.9 | 64 | 10 | 7 - 12 |

*The number of mutants represents the total number of induced and spontaneous mutations for caffeine resistance.

**Frequency of spontaneous mutants in the wild type strain.

the 65% survival level and a levelling off at the 20% survival. At survival levels greater than 13% the uv-induced mutations appeared to accumulate at a faster rate in gs-3 when compared to wild type (Fig. 12). Both, mutant gs-3 and wild type reached a plateau for mutation yield at comparable survival levels (16-20%). Fewer mutations were produced in gs-20 when compared to gs-6 over the survival range of 50-5%. However, the actual difference proved not to be statistically significant (Table IV). At a survival rate of 2%, mutant gs-20 continued to accumulate caffeine resistant mutations whereas at the same level of survival gs-6 produced no mutation. At higher survival levels(greater than 40%) mutant gs-1 accumulated caffeine resistant mutations at a faster rate than that of the wild type strain (Fig. 13). Followed by a short plateau a further increase in yield was observed for gs-1 at lower survival levels ( below 9%).

The mutation curve of strain gs-2 approximately paralleled that of mutant gs-1 at survival levels below 20%. Unlike the response of strain gs-1, the mutation yield in gs-2 was found to be lower. At a survival rate of 12% the reduction in mutation yield of gs-2 was found not to be statistically significant at the 5% significance level (Table IV) as compared to the mutation yield obtained at the 22% survival rate. At lower survival rates (below 10%) the mutation curve of gs-2 regained the slope of the mutation curve of mutant gs-1. At all survival levels mutant gs-2 produced fewer caffeine resistant mutations than gs-1. Like wild type,mutant gs-4 (Fig. 13) showed a steady increase in the yield of caffeine resistant mutation at higher survival level (above 50 %) followed by a sharp increase in the yield until 20% survival was reached. At 20% survival a short plateau in mutation yield was established. This plateau was

followed by a further increase in the mutation frequency causing the mutation curve of gs-4 to parallel the mutation curves of gs-1 and gs-2.

Hence, whether the comparison between uv-mutability of the wild type strain pe (Y8743m)a and gamma-ray-sensitive mutants is made on the basis of uv-dose (Figs. 10 and 11) or on the incidence of survival (Figs. 12 and 13), it is evident that more mutations to caffeine resistance were induced by uv-light in the wild type strain than in the gamma-ray-sensitive mutants, gs-1, gs-2, gs-3, gs-4, gs-6 and gs-20.

The effect of photoreactivation on the yield of uv-induced mutations for caffeine resistance was studied in the wild type strain pe (Y8743m)a. A significant reduction in the frequency of forward mutations for caffeine resistance was observed as a result of photoreactivation following uv-treatment especially at survival levels greater than 20% (Fig. 11, Table VI).

TABLE VI. Frequency of spontaneous and uv-induced caffeine resistant mutants in the wild type strain pe (Y8743m)a of N. crassa in the dark and after visible light treatment (photoreactivation).

| uv-dose in minutes | Survival (%) | | Caffeine resistant mutants per $10^7$ survivors | | | |
|---|---|---|---|---|---|---|
| | | | Observed | | Expected with 95% probability | |
| | Dark | Light | Dark | Light | Dark | Light |
| 0 | 100 ± 5.6 | 100 ± 4.8 | 19 | 9 | 14-25 | 2-27 |
| 1 | 86.4 ± 4.3 | 95.0 ± 7.2 | 39 | 9 | 32-4 | 3-21 |
| 3 | 21.5 ± 1.7 | 25.8 ± 2.0 | 316 | 141 | 269-371 | 77-237 |
| 6 | 1.1 ± 0.1 | 4.4 ± 0.2 | 407 | 303 | 286-561 | 157-529 |

FIG. 14. Back mutation rate (ascending curves) to adenine independence and per cent survival (descending curves) of wild type strain, (W.T.) strain, $gs^{+}$; *pan*-2 (B5), *ad*-1, *A* and gamma-ray sensitive mutants, *gs*-4, *pan*-2 (B5), *ad*-1, *A* and *gs*-6, *pan*-2(B5), *ad*-1, *A* plotted as a function of uv-dose (dose rate: 11.3 ergs $mm^{-2}$ $sec^{-1}$).

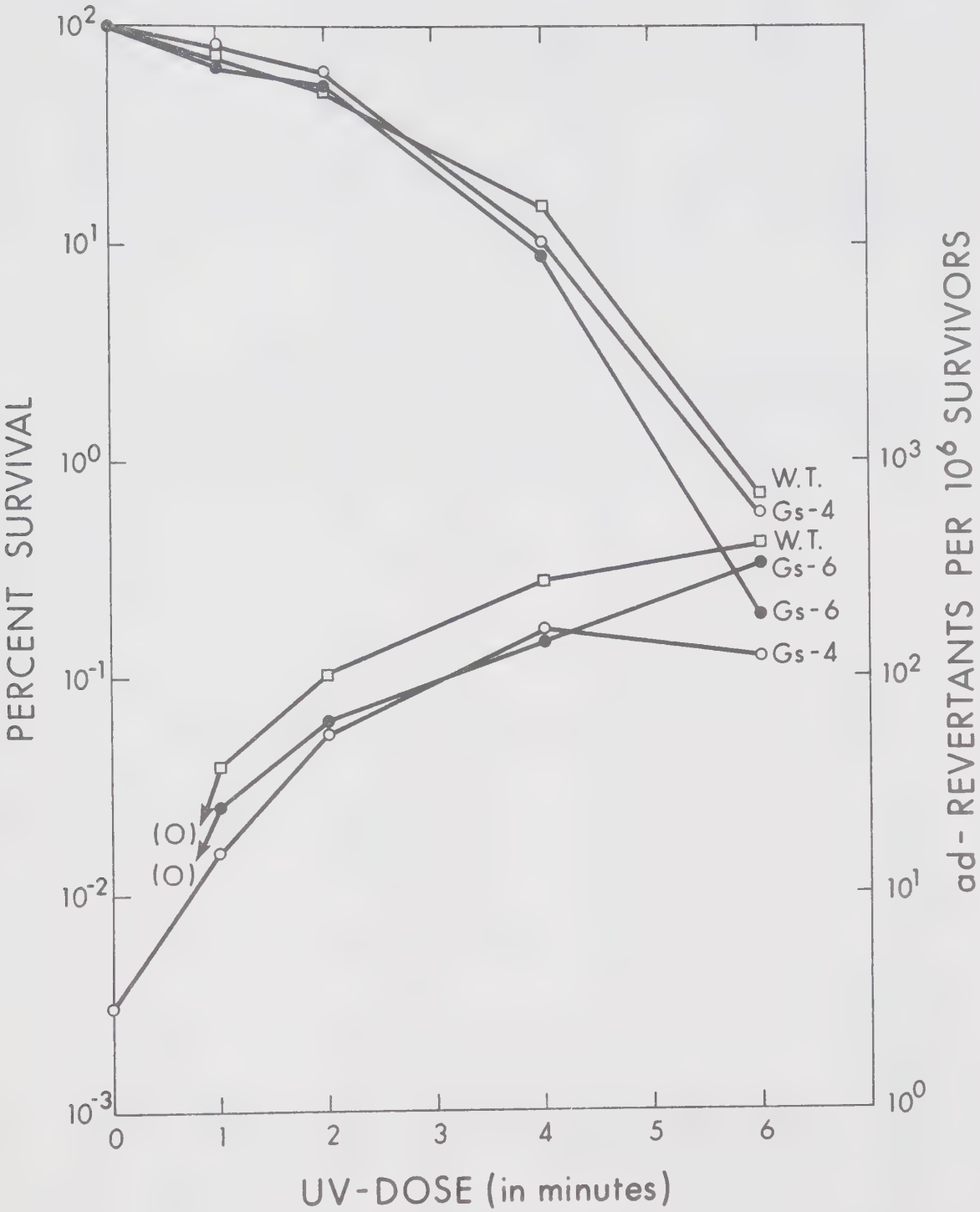

PERCENT SURVIVAL
ad- REVERTANTS PER $10^6$ SURVIVORS
UV-DOSE (in minutes)
W.T.
Gs-4
W.T.
Gs-6
Gs-6
Gs-4
(O)
(O)
$10^2$
$10^1$
$10^0$
$10^{-1}$
$10^{-2}$
$10^{-3}$
$10^3$
$10^2$
$10^1$
$10^0$
0
1
2
3
4
5
6

TABLE VII. Frequency of spontaneous and uv-induced *ad*$^+$ revertants in wild type and gamma-ray sensitive mutants of *N. crassa*.

| Strain | uv-dose in (Min.) | Survival (%) | Frequency of ad$^+$- revertants per $10^6$ survivor | |
|---|---|---|---|---|
| | | | Observed | Expected with 95% probability. |
| Wild type *gs*$^+$ | 0 | 100.0 ± 9.7 | 0 | 0 - 4 |
| *pan*-2(B-5) | 1 | 72.0 ± 5.9 | 39 | 29 - 51 |
| ad-1A | 2 | 50.0 ± 3.7 | 105 | 85 - 129 |
| | 4 | 15.5 ± 0.6 | 284 | 225 - 353 |
| | 6 | 0.7 ± 0.1 | 425 | 138 - 992 |
| *gs*-4, *pan*-2(B-5) | 0 | 100.0 ± 7.4 | 3 | 1 - 7 |
| *ad*-1 | 1 | 81.7 ± 8.7 | 16 | 11 - 24 |
| | 2 | 63.4 ± 3.2 | 55 | 43 - 70 |
| | 4 | 10.0 ± 1.7 | 164 | 106 - 242 |
| | 6 | 0.6 ± 0.1 | 123 | 3 - 683 |
| *gs*-6,*pan*-2 (B5) | 0 | 100.0 ± 12.0 | 0 | 0 - 3 |
| *ad*-1 | 1 | 67.4 ± 0.6 | 25 | 17 - 36 |
| | 2 | 50.9 ± 1.3 | 62 | 47 - 81 |
| | 4 | 9.2 ± 0.5 | 151 | 96 - 227 |
| | 6 | 0.2 ± 0.02 | 347 | 8 - 1934 |

(ii) Acriflavine Resistant Mutations:

Forward mutations for acriflavine resistance were induced by uv-light in wild type strain pe (Y8743m)a and three gamma-ray sensitive mutants, gs-3, gs-6 and gs-20 (Table IV). It is apparent from the results that the yield of mutations to acriflavine resistance remained extremely low in all the strains tested. No acriflavine resistant mutant was recovered in strain gs-20 whereas the incidence of mutations in wild type gs-3 and gs-6 was extremely low. Due to this low incidence of uv-induced forward mutations for acriflavine resistance together with the high degree of inconsistency in the results, no attempt was made to induce mutations for acriflavine resistance in other gamma-ray sensitive mutants.

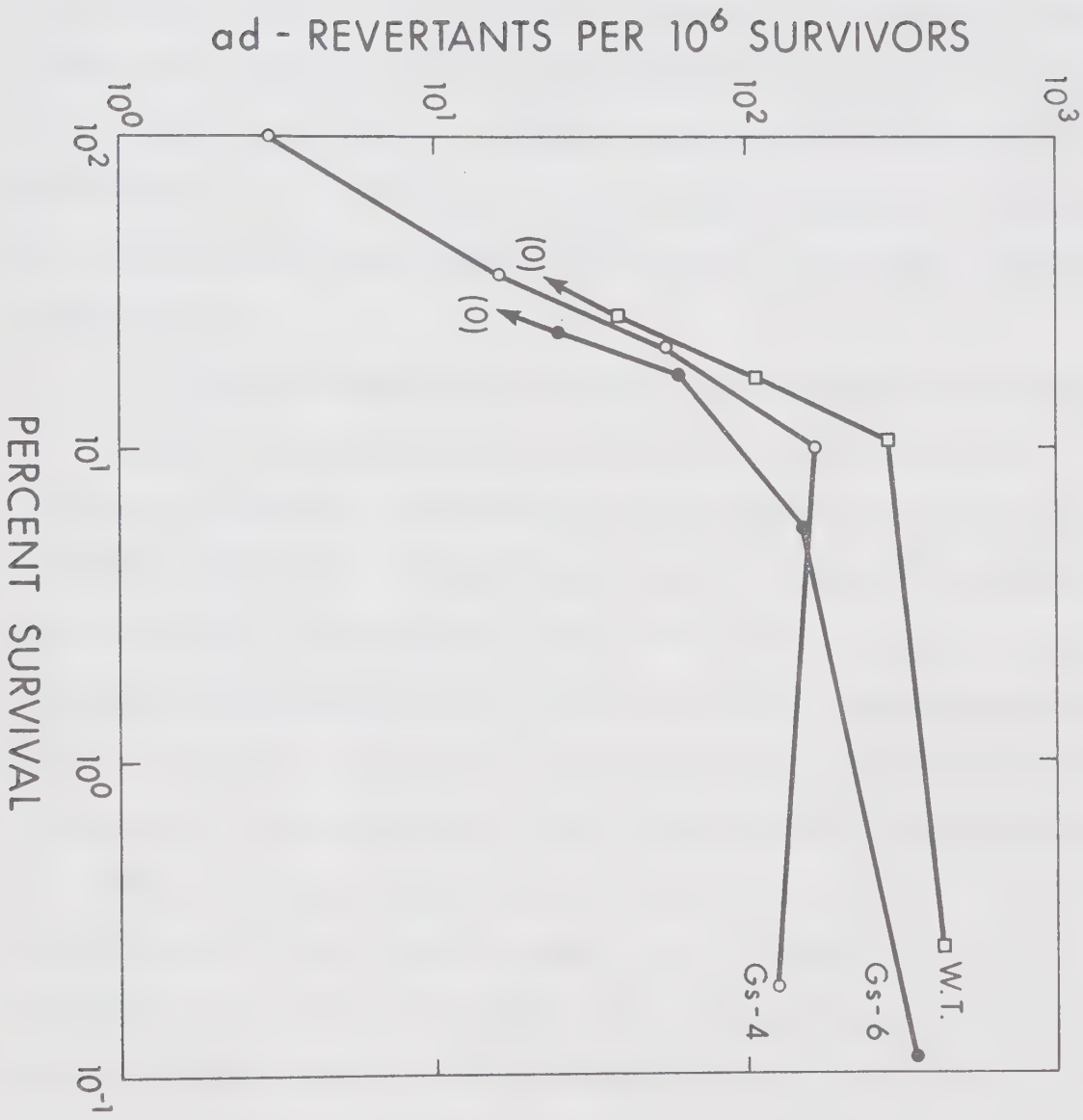
(iii) $ad^+$ Revertants:

Two of the gamma-sensitive mutants, gs-4 and gs-6 which showed increased sensitivity to gamma-rays and produced fewer uv-indiced mutations for caffeine resistance, were further used for the studies of uv-induced mutability for $ad^+$ revertants. The ad-1 marker was introduced in the mutants, gs-4, gs-6 and wild type strains as described before. The resulting strains were: $gs^+$, pan-2(B5), ad-1 A; gs-4 pan-2(B5), ad-1 A and gs-6 pan-2(B5) ad-1 A.

The frequency of uv-induced adenine revertants was lower in mutants gs-4, pan-2 (B5), ad-1A and gs-6, pan-2 (B5) ad-1 A when compared to wild type $gs^+$ pan-2 (B5) ad-1 A over the entire uv-dose used (Fig. 14, Table VII).

No spontaneous revertants were recovered in the wild type ($gs^+$) and mutant gs-6 in these experiments. The mutation curve of the wild type strain ($gs^+$) showed a steady increase in the yield of $ad^+$ revertants

FIG. 15. uv-induced back mutation rate to adenine independence of a wild type (W.T.) strain, gs$^+$, pan-2 (B5), ad-1, A and of gamma-ray sensitive strains, gs-4, pan-2 (B5), ad-1, A and gs-6 , pan-2 (B5), ad-1, A as a function of percent survival.


ad - REVERTANTS PER $10^6$ SURVIVORS
$10^0$
$10^1$
$10^2$
$10^3$
PERCENT SURVIVAL
$10^2$
$10^1$
$10^0$
$10^{-1}$
(0)
(0)
W.T.
Gs-6
Gs-4

with increasing uv-dose. At uv-dose levels <2712 ergs $mm^{-2}$ the mutation curves of gs-4 and gs-6 paralleled approximately the mutation curve of wild type control, indicating a similar rate of prototroph production in the three strains. At higher uv-dose levels (>2712 ergs $mm^{-2}$) however, mutant gs-6 continued to accumulate uv-induced $ad^+$ revertants, whereas mutant gs-4 appeared to show a slight decline in the mutation frequency at the same dose levels. At the dose range lower than 2712 ergs $mm^{-2}$ the frequency of uv-induced $ad^+$ revertants was significantly reduced in both the gamma-sensitive mutants as compared to wild type (at 5% level of significance).

When the frequency of uv-induced $ad^+$ revertants was plotted as the function of survival (Fig. 15), it became evident that fewer mutations per survivor were induced in gamma sensitive mutants, gs-4 and gs-6 than in the wild type strain ($gs^+$). It should be pointed out that the shape of the mutation curves of the wild type ($gs^+$) strain for uv-induced $ad^+$ revertants and for caffeine resistant mutations are quite similar (Figs.12, 13,15). However mutants gs-4 and gs-6 exhibit different responses for the uv-induction of $ad^+$ revertants and forward mutations for caffeine resistance especially at lower survival levels. At the survival rates lower than 10% mutant gs-4 continued to accumulate caffeine resistant mutations whereas the frequency of $ad^+$ revertants showed a gradual decline at these survival levels. Mutant gs-6 on the other hand, showed no mutation for caffeine resistance at lower survival levels (<20%), whereas there was a continuous increase in the yield of $ad^+$ revertants at these survival rates.

It is obvious that the shapes of the uv-induced mutation curves in all the strains described above are complex, which probably reflects the complexity of the process of uv-mutagenesis itself and also the nature of the block in the DNA repair pathways caused by a specific mutation carried by an individual gamma-sensitive strain. The possibility that several other factors are involved in affecting the shape of uv-dose effect curves for mutations, cannot be ruled out (Auerbach and Kilbey, 1971).

Despite the complex ultraviolet dose-effect curves for mutations, it is evident that fewer uv-induced mutations are produced per survivor in all gamma-sensitive strains when compared to their wild type progenitor. Assuming that an equal number of premutational uv-lesions are produced in wild type and gamma-ray-sensitive mutants, it appears that most of such lesions failed to produce detectable mutations in gamma-sensitive mutants.

Interallelic Recombination:

To investigate the effect of increased sensitivity to gamma-rays on genetic recombination , inter-allelic recombination between two pan-2 alleles, (pan-2 ($B_3$) and pan-2 (B5) was studied in crosses homozygous for the mutants, gs-2, gs-4, gs-6 and gs-20 and compared with the prototroph frequency between the same pan-2 alleles but in crosses homozygous for the wild type allele $gs^+$ for gamma-ray resistance. All crosses were fully fertile, i.e., produced normal perithecia and shed spores in abundance except the cross involving mutant gs-6 in homozygous condition, which produced approximately 60% immature brown perithecia without bearing any spores; however, the remaining normal perithecia from this cross produced abundant spores. Viability of ascospores ranged from 50 - 80% in all the crosses.

FIG. 16. Frequencies of *pan*$^+$ prototrophs (per $10^6$ viable ascospores) from crosses homozygous for wild type (*gs*$^+$), *gs*-2, *gs*-4, *gs*-6, and *gs*-20. Each dot represents the frequency of *pan*$^+$ prototrophs colonies from one crossing plate. The open circles represents the average prototroph frequency from each cross.

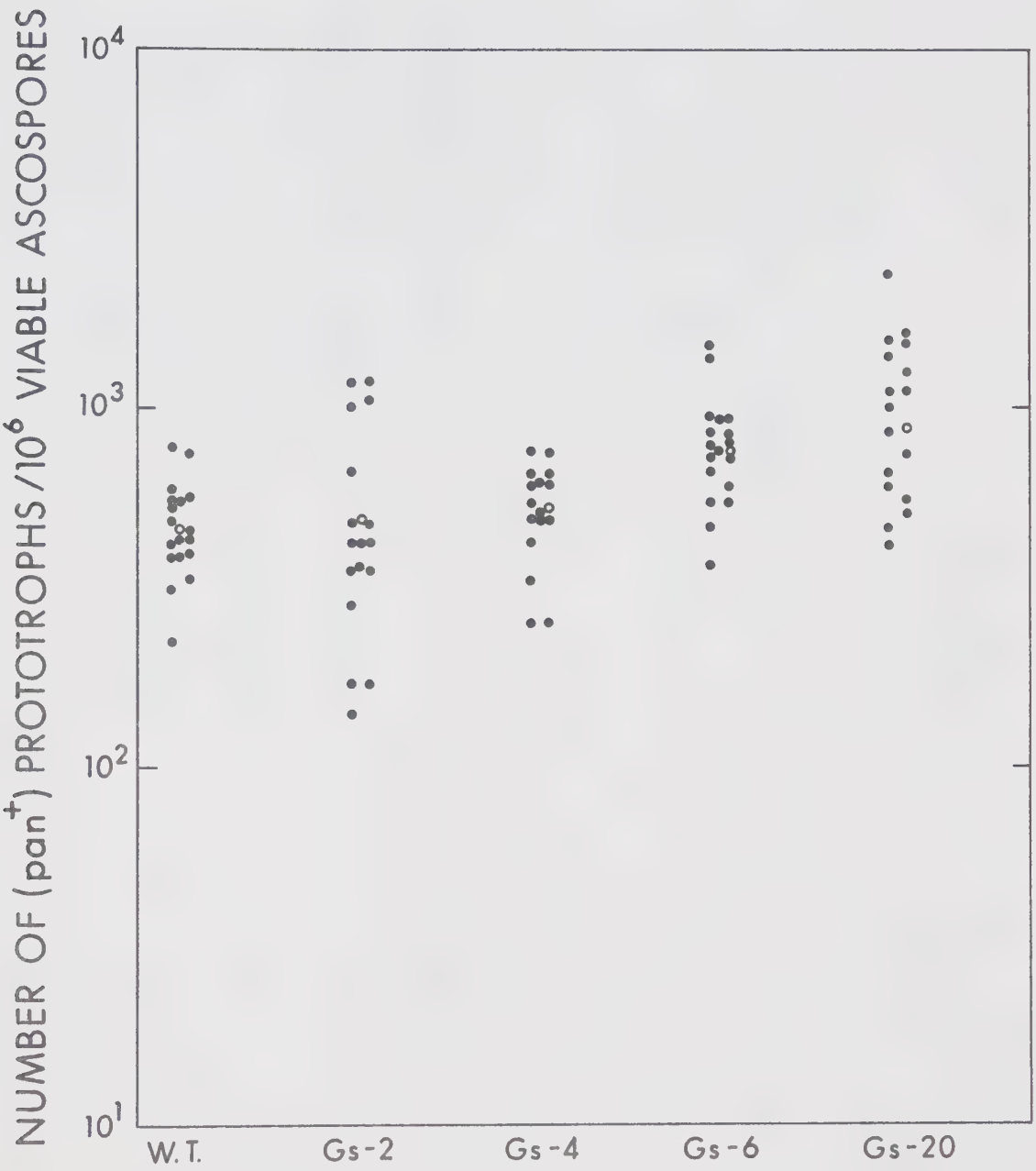

NUMBER OF (pan+) PROTOTROPHS /10^6 VIABLE ASCOSPORES
10^4
10^3
10^2
10^1
W.T.
Gs-2
Gs-4
Gs-6
Gs-20

TABLE VIII. Effect of gamma-ray sensitive mutants, gs-2, gs-4, gs-6 and gs-20 on recombination between alleles, pan-2(B5) and pan-2-(B3) in Neurospora crassa.

| Cross | No. of $pan^+$/ No. of viable spores | No. of $pan^+/10^6$ viable spores | |
|---|---|---|---|
| | | Observed | Expected with 95% probability |
| Controls, ($gs^+$) | | | |
| 46-5 al -2, pan-2(B3), Tryp-2,A x 467 pan-2(B5), ad-1a | 178/309,652 | 574 | 494 - 666 |
| al-2, pan-2(B3), tryp-2a x pan-2(B5), ad-1A | 406/903,387 | 449 | 407 - 496 |
| Homozygous (gs) | | | |
| gs-2,pan-2(B3)tryp-2a x gs-2, pan-2(B5), ad-1A | 83/172,800 | 480 | 383 - 596 |
| gs-4, pan-2(B3), tryp-2a x gs-4,pan-2(B5), ad-1A | 302/571,621 | 528 | 470 - 592 |
| gs-6, pan-2(B3) al-2,tryp-2A x gs-6, pan-2(B5), ad-1A | 505/676,267 | 747 | 680 - 815 |
| gs-20, pan-2(B3), tryp-2A x gs-20,pan-2B5, ad-1a | 441/465,993 | 882 | 860 - 1039 |

The frequency of pan-2 prototrophs in all the crosses studied showed a large degree of variation (Fig. 16). The average prototroph ($pan^+$) frequency and frequency expected with 95% probability were calculated for each cross (Table VIII).

These results show that the frequency of $pan^+$ recombinants in the crosses homozygous for gs-2 and gs-4 is not significantly different from the one obtained from the control crosses. However, crosses homozygous for gs-6 or gs-20 produced significantly higher frequencies of $pan^+$ prototrophs when compared with the control crosses. The control crosses differed in the frequency of $pan^+$ recombinants, but this difference was not found to be statistically significant.

# DISCUSSION

Genetics, isolation and characterization of gamma-ray sensitive mutants.

Six gamma-ray sensitive mutants: gs-1, gs-2, gs-3, gs-4, gs-6 and gs-20, were isolated in Neurospora crassa (Plate I). Genetic tests indicated that the radiation sensitivity of each of these mutants is under the control of a single nuclear gene (Plate II and III; Table II). Since in general, an increase in the number of nuclei per cell sometimes enhances the resistance of the cell to radiation treatment (Clark, 1968), radiosensitivity might be caused by a decrease of the nuclear content of a multinucleate cell. However,all the mutants when compared to wild type, showed a quantitatively similar distribution of nuclear numbers per conidium and hence it is evident that the gamma-ray sensitivity of these strains is not due to a mutation which gives rise to a decrease in the average number of resident nuclei in the conidium.

From the comparison of dose response curves (Figs. 1 and 2) and dose modifying factors (Table I) it is clear that the six gamma-ray sensitive mutants differ in their sensitivity to gamma radiation. Both, genetical as well as biochemical studies (Strauss, 1968; Howard-Flanders, 1968) have established the fact that sensitivity to radiation (uv and ionizing radiation) and/or chemical mutagens is usually caused by the impairment of enzyme mediated DNA repair processes of the cell. Thus, it is reasonable to assume that the enhanced gamma-ray sensitivity of the six mutants reported on here, is due to the mutation of genes controlling dark repair processes in Neurospora crassa. However, manifestation of changes at the ultrastructural level such as swelling

of cell wall and mitochondria in gamma-ray irradiated conidia and mycelia (grown from irradiated conidia) as reported for mutant gs-3, may have contributed to the increase of gamma-ray sensitivity (in terms of conidial inactivation) since little or no such ultrastructural changes were observed in the wild type strain (Plate 4 and 5; Table III).

The variable response to gamma-ray inactiviation as exhibited by the gamma-ray sensitive mutants is presumably due to the varying phenotypic expressions of the genes involved in the control of different steps of the repair pathways. Nonetheless, other factors such as a reduction of membrane permeability, secondary mutations as well as others as the result of radiation treatment, can not be ruled out as a possible source for the observed variability in the response of these mutants to ionizing radiation.

The dose response curve of mutant gs-6 is markedly different from the other gamma-ray sensitive mutants (Fig. 2). The final slope of its survival curve is however not very different from the one obtained with the wild type strain which suggest the possibility of the existence of dose dependent gamma-ray repair pathways. As postulated by Cox (1968), a survival curve similar to the one observed for mutant gs-6 can be expected when a repair system functioning at low dose levels is impaired and one operating at higher radiation doses is still intact. besides being sensitive to gamma rays, mutant gs-6 is also sensitive to the lethal effects of uv-light (Fig.10). Mutants sensitive to uv and gamma-rays have also been reported in yeast, S. cerevisiae. The gamma-ray dose-response curves for the survival of some of these mutants (Cox and Parry, 1968) are comparable to the survival curves obtained for mutant gs-6.

Photoreactivation treatment increased the survival of uv-irradiated conidia of the wild type strain and mutant gs-6, the latter being sensitive to both gamma-rays and uv-light (Figs. 5 and 6). This observation suggests that pyrimidine dimers are involved in the inactivation of conidia of N. crassa when irradiated with uv light, a conclusion already reached at by Kilbey and DeSerres (1967). The earlier findings that Neurospora crassa possesses photoreactivating enzyme (Terry and Setlow, 1967; Terry et al, 1967) which is known to split the pyrimidine dimers (Wulff and Rupert, 1962) corroborates this suggestion. More recently Tuveson and Mangan (1970) have provided genetic information for the existence of photoreactivation by isolating a mutant of N. crassa defective in this process.

From bacterial studies it is known that when uv-irradiated bacterial cells are held in buffer before plating on the plating medium the survival of the cells is increased (Harm, 1968) by excision repair (Setlow and Carrier, 1964). "Liquid holding recovery" after uv-irradiation has also been observed in yeast (Patrick, Haynes and Uretz, 1964; Parry and Parry, 1969 Hunnable and Cox, 1971). However, in Neurospora the effect of liquid holding appears to be complicated. The results of the present study show that the wild type strain pe (Y8743m)instead of recovery of uv-irradiated conidia, exhibited a consistent decline in the survival after liquid holding treatment when compared with the survival after immediate plating (Fig. 5). A similar negative liquid holding effect after uv-irradiation was reported by Schroeder (1970b)for wild type Neurospora crassa and by Harm and Haefner (1968) for wild type strains of Schizosaccharomyces pombe and E. coli C.

Radiation sensitive mutants, on the other hand, produced a variable response to liquid holding treatment. Mutant gs-6 of Neurospora crassa showed a negative liquid holding effect at all dose levels except at the

uv-dose of 678 ergs $mm^{-2}$ at which level of irradiation liquid holding recovery can be observed (Fig.6). Like wild type, the negative liquid holding effect in gs-6 increases with increasing dose. The above data differs from that reported on by Schroeder (1970b) that radiation sensitive mutants of N. crassa, unlike the wild type (i.e., characterized by dose dependent negative liquid holding effect) displayed liquid holding recovery over the entire uv-dose range. Either a negative liquid holding effect or a liquid holding recovery was described to occur in radiation sensitive mutants of the yeasts S. cerevisiae (Parry and Parry, 1969) and S. pombe (Fabre, 1970; Harm and Haefner, 1968). These findings together with those reported on here indicate that the response of some Eukaryotes (e.g., yeasts and Neurospora) to liquid holding treatment is more complex than observed in similar studies with Prokaryotes. There is no doubt however, that both the negative liquid holding effect and the liquid holding recovery are associated with the dark repair processes operating in yeasts, Neurospora as well as in bacteria. Consequently, the liquid holding response of the wild type (radio-resistant) strains of these organisms is frequently altered by mutations which in addition give rise to radiation sensitivity (Schroeder, 1970; Fabre, 1970; Harm and Haefner, 1968; Parry and Parry, 1969). The mechanisms underlying the processes of liquid holding recovery and negative liquid holding together with their interrelationship, awaits further investigation.

The survival of uv-irradiated conidia after delayed photoreactivation remained unaffected in the wild type strain of N. crassa (Fig. 5) suggesting that photoreactivable uv-lesions were either removed or altered by dark repair processes operating prior to photoreactivation

treatment when the conidia are held in liquid. Similar observations have been reported by Harm (1968) for bacteria, by Parry and Parry (1969) for S. cerevisiae, and by Schroeder (1970b) for N. crassa. The present mutant gs-6, on the other hand, showed a small but consistent increase in survival after delayed photoreactivation (Fig. 6) and parallels in this aspect several radiation sensitive mutants of Neurospora (Schroeder, 1970b) and Saccaromyces (Parry and Parry,1969). Mutant gs-6 differs from others in that at the uv-dose of 678 ergs $mm^{-2}$ delayed photoreactivation treatment gives higher survival than immediate photoreactivation treatment. As pointed out above the uv-dose of 678 ergs $mm^{-2}$ also results in a liquid holding recovery for this mutant rather than a negative liquid holding effect (which is characteristic for mutant gs-6 at higher dose levels of irradiation ). Both these observations taken together suggests that at the uv-dose of 678 ergs $mm^{-2}$ the additive effects of liquid holding recovery and photoreactivation, together with the absence of a negative holding effect, can be held responsible for the increased survival after delayed photoreactivation in mutant gs-6. However, the possible involvement of several other unknown factors affecting the survival of conidia after post-irradiation treatments can not be ruled out. The enhancement of conidial survival after delayed photoreactivation as observed for mutant gs-6, indicates that this mutation at least partly affects the excision of photoreactivable uv-lesions.

Caffeine is known to impair dark repair processes in different organisms, both Prokaryotes and Eukaryotes (Witkin, 1969; Patrick and Haynes, 1964; Lumb et al., 1968: Fabre, 1970). The present investigation reveals that caffeine plays a rather similar role in N. crassa.

uv-irradiated conidia of a caffeine resistant strain when plated on a medium containing caffeine showed a decreased survival as compared with the survival levels obtained on plates free of caffeine (Fig. 9). Assuming that post-irradiation treatment with caffeine should not have any effect on the survival of a strain defective in dark repair processes, the conidia of mutant gs-6 and wild type (control) strain were held in caffeine solution after irradiation and prior to plating on a caffeine-free plating medium. The results indicated no significant change in the survival of conidia of the wild type as well as of mutant gs-6 due to the treatment with caffeine. In comparing these results with those obtained with uv-irradiated conidia of a caffeine resistant strain plated onto a plating medium containing caffeine (see above), it is evident that caffeine is effective only when present in the post-irradiation plating medium and not when present in holding solution prior to plating (Figs. 7 and 8). A concentration of 0.25% of caffeine in the plating medium is sufficient to cause no survival of both wild type and mutant gs-6. In comparing these results with those obtained with caffeine present in the post-radiation treatment prior to final plating in the absence of caffeine, it becomes clear that the germinating conidium is susceptible to caffeine whereas the resting conidium is not affected by it. Caffeine present in an immediate post-irradiation treatment (caffeine in distilled water) of non-germinating (i.e., resting) conidia does not influence the survival of these conidia whereas the presence of caffeine during the initiation of the vegetative cycle of the fungus will lead to death of the developing organism. Hence, the post-irradiation treatment with caffeine does not provide results which

TABLE IX. A comparison of the properties of radiation sensitive mutants of *E. coli*, *S. cerevisiae* and *N. crassa*.

| | Phenotype | Sensitivity to X-ray/gamma-rays | uv | uv-induced mutability | Genetic recombination | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Meiotic | | Mitotic | | |
| | | | | | Inter genic | Intra genic | Inter genic | Intra genic | |
| *E. coli* | *Exr*$^-$ | S | S | L | | | L | | 1) |
| | *rec*$^-$ | S | S | L | | | L | | 1) |
| | *hcr*$^-$ | R | S | H | | | N | | 1) |
| *S. cerevisiae* | *uvs* | - | S | - | N | N | H* | N,H* | 2) |
| | *uvs* | R | S | H | N | N | - | - | 3,4,5) |
| | *uvs* | S | S | H | - | - | - | - | 4) |
| | *uvs* | S | S | L | - | - | - | - | 4) |
| | *X-s* | S | R | - | sterile | | - | - | 5) |
| | *X-s* | S | R | - | sterile | | L | - | 6) |
| | *rec*$^-$ | S | R | - | sterile | | N* | L* | 7,8) |
| | *rec*$^-$ | S | S | - | - | - | - | L* | 7) |
| | *rev*$^-$ | S | S | L | N | N | H* | H* | 9) |
| *N. crassa* | *uvs* | - | S | L | N | - | - | - | 10,11) |
| | *uvs* | - | S | H | N | N | - | - | 11,12) |

TABLE IX cont'd.

| | Phenotype | Sensitivity to X-ray/gamma-rays | uv | uv-induced mutability | Genetic recombination | | | | References |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Meiotic | | Mitotic | | |
| | | | | | Inter genic | Intra genic | Inter genic | Intra genic | |
| *N. crassa* cont'd | *uvs* | S | S | L | sterile | | - | - | 11,13) |
| | *uvs* | - | S | L | - | N | - | - | 11,13) |
| | *uvs* | - | S | L | sterile | | - | - | 11,13) |
| | *uvs* | - | S | L | - | - | - | - | 11,14) |

Abbreviations: S = sensitive; R = resistant; H = high; L = low; N = normal; * = radiation induced response.

(-) = not known; *uvs* = uv-sensitive; *rec*$^-$ = recombination deficient; *rev*$^-$ = reversion less;

*X-s* = X-ray sensitive; *hcr*$^-$ = excision defective; *Exr*$^-$ = sensitive to X-rays.

References:

1) Witkin (1969
2) Snow (1968)
3) Nakai and Yamaguchi (1969)
4) Averbeck *et al.* (1970)
5) Resnick (1969)
6) Nakai (1969)
7) Rodarte-Ramon and Mortimer (1972)
8) Rodarte-ramon (1972)
9) Lemontt (1971)
10) Chang, Lennox and Tuveson (1968)
11) De Serres (1971)
12) Stadler and Smith (1968)
13) Schroeder (1970a)
14) Tuveson and Mangan (1970)

can be taken as conclusive evidence for the existence of a defective dark repair process in mutant gs-6 of Neurospora crassa.

uv-induced mutability and genetic recombination.

The mutants of E. coli defective in genetic recombination produce no or very few uv-induced mutations, suggesting that the processes of genetic recombination and uv-mutagenesis probably involve similar enzymatic steps (Witkin, 1969). E. coli recombination deficient mutants are sensitive to both, uv- and ionizing-radiation (X-rays or gamma-rays). It is understood that this sensitivity is due to a defect in a dark repair process, called post-replication repair or recombinational repair mechanism (Rupp and Howard-Flanders, 1968). Mutants of another type which are defective in excision repair due to their inability to excise uv-induced lesions of DNA, are sensitive to uv-light and not to ionizing radiation (Howard-Flanders, 1968). These mutations enhance uv-mutability and are normal in their response to recombination (Witkin, 1969).

To extend these studies to Eukaryotes various radiation sensitive mutants have been isolated from different organisms and a limited number of these mutants were subjected to recombination and uv-mutagenesis studies (Strauss, 1968; Resnick, 1969; Nakai, 1969; Avenbeck et al., 1970; Fortuin, 1971b; Schroeder, 1970a; De Serres, 1971; Fogel and Mortimer, 1971). Properties of radiation sensitive mutants of E. coli, S. cerevisiae and N. crassa with respect to their response to genetic recombination and uv-mutability are summarized in Table IX.

In comparing the properties of radiation sensitive mutants of yeast and Neurospora the following classification with regared to their uv-mutability and recombination can be made: (i) yeast mutants with

reduced uv-mutability are all sensitive to uv- and ionizing radiation and where tested, showed normal recombination; (ii) Neurospora mutants defective in uv-mutability are all sensitive to uv and are either sterile or normal in their response to genetic recombination, where tested, these mutants were found to be sensitive to ionizing radiation; (iii) yeast mutants sensitive to uv and with an increased rate of uv-induced mutability but normal in their response to genetic recombination; (iv) Neurospora uv-sensitive mutants characterized by an increased yield of uv-induced mutations are all normal in genetic recombination. It is evident from the above, that the reduction in uv-mutability in mutants of both yeast and Neurospora is not associated (as in E. coli) with the deficiency in genetic recombination and hence in these Eukaryotes uv-mutagenesis and genetic recombination are as processes independent of each other. Nevertheless, several mutants of Neurospora and yeast are known which have uv-mutability and recombination characteristics similar to the exision defective mutants of E. coli. The disparity between radiation sensitive mutants of E. coli and N. crassa with regard to their response to genetic recombination and uv-mutability is also evident from the results of the present investigation. Gamma-sensitive mutants of N. crassa were tested for uv-mutability and genetic recombination. All of the six gamma-ray sensitive mutants gs-1, gs-2, gs-3, gs-4, gs-6 and gs-20 yielded fewer uv-induced forward mutations for caffeine resistance than their wild type progenitor (Figs. 10 and 11). uv-mutability for back mutation to adenine prototrophy was studied in mutant gs-4 and gs-6. Both of these mutant strains showed a reduction in the frequency of $ad^{+}$ prototrophs when compared to the wild type strain (Fig. 14).

Although quantitatively all gamma-ray sensitive mutants showed a reduction in the yield of uv-induced mutations, qualitatively the uv-mutability response appeared more complex as reflected by the variable shapes of the mutation curves for the individual strains. It appears that the complexity of the process of uv-mutagenesis itself and the nature of the block in the DNA repair pathways caused by the mutation to gamma-ray sensitivity, are the main factors influencing the shape of the uv-dose effect mutation curves of these strains. In addition, Auerbach and Kilbey (1971) list several other factors which may contribute to the complexity of the uv-induced mutation curves.

Assuming that at a given dose level the average number of pre-mutational uv-lesions per survivor are similar in wild type and gamma-ray sensitive mutants, it can be predicted that the number of detectable uv-induced mutations per survivor should be comparable regardless as to whether the strain is gamma-sensitive or resistant. Contrary to this prediction it was found that all gamma-ray sensitive strains produced fewer uv-induced mutations per surviving cell when compared with the wild type(Figs. 12,13 and 15) which suggests that in gamma-ray sensitive mutants most of the pre-mutational lesions fail to produce detectable mutations. It therefore appears that gamma-ray sensitive mutants are defective in the process of uv-mutagenesis.

The presence of caffeine in the post-irradiation plating medium (as used for the selection of caffeine resistant mutants) is not implicated in the reduced uv-mutability as exhibited by the gamma-ray sensitive strains. In general, the presence of caffeine enhances the lethal and mutagenic effects of uv-light in both Pro- and Eukaryotes,

presumably by impairing the dark repair processes (Grigg, 1968; Witkin, 1969; Trosko and Chu, 1971; Moustacchi and Enteric, 1970; Lumb et al., 1968; Fabre, 1970). Similarly, when present in the post-irradiation plating medium, caffeine was found to enhance the lethal effects of uv-light in caffeine resistant mutants of N. crassa (Fig. 9), implying that caffeine inhibits the dark repair process and probably increases the mutagenic effect of uv. As is evident from the low levels of uv-induced mutation rates the enhancement of the mutagenic effect of uv by caffeine is suppressed in all the gamma-ray sensitive mutants tested.

Statistically, the yield of uv-induced mutations is significantly reduced ($p=0.05$) over almost the entire uv-dose range used for all the gamma-ray sensitive mutants except in mutant gs-3 which, when tested over the same dose range, showed only a small reduction in the uv-induced mutation rate (Table IV).

Electron microscope studies of mutant gs-3 indicate that radiation sensitivity of this mutant is presumably affected in cellular processes other than those associated with the DNA repair processes. It is therefore possible that the uv-mutability of mutant gs-3 has been reduced as a result of the inherit increased wall thickness of this isolate when compared to the wild type strain (Table III). From literature it is known that in several organisms cell wall thickness alters the uv-induced mutation frequency (Auerbach and Kilbey, 1971).

Interallelic recombination was studied in gamma-ray sensitive mutants gs-2, gs-4, gs-6 and gs-20. Each of the mutant (gs) or wild type allele ($gs^+$) when in homozygous condition showed a wide range

in the frequency of *pan*$^+$ prototrophs produced as a result of recombination between two *pan*-2 alleles: *B 3* and *B 5* (Fig. 16). No significant difference in the *pan*$^+$ prototroph frequency was observed in mutants *gs*-2 and *gs*-4 when compared to wild type, however, mutant *gs*-6 and *gs*-20 produced a significantly higher frequency of interallelic recombination (Table VIII). Since the observed magnitude of variation amongst the different crosses is not unusual for *Neurospora* (Schroeder, 1970a), the conclusion that the four mutations *gs*-2, *gs*-4, *gs*-6 and *gs*-20 conferring gamma-ray sensitivity do not have any effect on inter-allelic recombination in *Neurospora*, seems to be warranted. Moreover, the recombination frequency between the two *pan*-2 alleles, B3 and B5 in all the crosses (Table VIII) is comparable to the value (0.1%) reported by Case and Giles (1958) for these two alleles.

Mutant *gs*-6 resembles the mutants of *S. cerevisiae* (Lemontt, 1971c) in its response to radiation sensitivity, uv-induced mutability and genetic recombination (Table IX). In this respect it is interesting to note that these yeast mutants were isolated directly for reduced uv-mutability. Schroeder (1970b) has also reported a mutant (*uvs*-3) of *N. crassa* with properties similar to those of mutant *gs*-6. Unlike mutant *gs*-6, crosses involving mutation *uvs*-3 in homozygous condition, were reported to be sterile. This sterility, however, was not associated with the process of meiotic recombination. Schroeder (*loc.cit.*) was able to demonstrate that the mutation *uvs*-3 causes an enhancement of the frequency of mitotic recombination.

The mutants *gs*-1, *gs*-2 and *gs*-20 of *N. crassa* belong to a class of mutants new to Prokaryotes as well as Eukaryotes. As discussed

above, these mutants are sensitive to ionizing radiation but not to ultraviolet light. They exhibit reduced uv-mutability and are normal in recombination. Although mutants sensitive to x-rays with normal sensitivity to uv-light have been isolated in S. cerevisiae, no data regarding their uv-mutability is as yet available (Table IX). Some of these yeast mutants exhibit reduced recombination or meiotic sterility.

The fact that the frequency of both, uv-induced forward mutation for caffeine resistance and $ad^+$ revertants was found to be reduced in mutant gs-4 and gs-6, suggests that the effect of these mutations on uv-mutability is perhaps general rather than locus specific as was shown by Chang et al., (1968) to be the case in Aspergillus nidulans: In this organism a uv-sensitive mutant yielded fewer uv-induced $ad^+$ revertants but the frequency of $met^+$ prototrophs remained normal when compared to a uv-resistant strain carrying the same markers. Recently, uv-sensitive mutants of N. crassa showing a reduced frequency of uv-induced forward mutation for caffeine resistance (Chang et al., 1968) and for adenine requirement (De Serres, 1971) have been reported.

It should be emphasized that mutant strains gs-4 and gs-6 vary in their response concerning uv-mutability of two different markers i.e., forward mutation for caffeine resistance and back mutations for adenine requirement (as is evident from the different shapes of the respective mutation curves, Figs. 10, 11 and 14). The shapes of the mutation curves of the wild type for these two markers (i.e., caffeine resistants and $ad^+$ revertants) are very much similar. Therefore, the different types of mutation together with the nature of the defect caused by a given mutation conferring radiation sensitivity, may account for the variability

observed with regard to uv-mutability of these gamma-ray sensitive strains. Nakai and Yamaguchi (1969) reported that uv-mutability response of a radiation sensitive mutant of S. cerevisiae was considerably influenced by the type of mutations (true back mutations, super-suppressors and addition-delection types) induced. They suggested that the dark repairability of pre-mutational damage depends upon the type of mutation. The possibility that the mutability response of a particular strain may also be variably affected by the different loci involved (Auerbach and Kilbey, 1971), can not be excluded.

Spontaneous mutability.

Besides reduction in uv-induced mutability, all gamma-ray sensitive mutants yielded fewer spontaneous mutations for caffeine resistance when compared with wild type (Table IV). Some of the radiation sensitive mutants of E. coli which showed reduced uv-mutability also suppressed spontaneous mutability, while others showed a normal yield of spontaneous mutations (Witkin,1969; Miura and Tomizawa, 1968). Similarly, in yeas mutations conferring radiation sensitivity have been reported to have increased the spontaneous mutation frequency (von Borstel,et al., 1968), whereas some radiation sensitive mutants showed a reduced spontaneous mutability (Averbeck et al., 1970). Different radiation sensitive mutants of N. crassa also showed a variable response for spontaneous mutability (De Serres, 1971). In the present study, no spontaneous mutations for adenine revertants were obtained in the wild type and mutant gs-6, whereas mutant gs-4 yielded a frequency of $^{3}$ revertants per $10^{6}$ survivors. These observations suggest that spontaneous mutagenesis is affected by the mutations causing radiation sensitivity, however, the interdependence of the process of spontaneous mutagenesis and DNA repair systems appears to be complex.

In summary, the results of the present investigation indicate that in N. crassa uv-mutagenesis and genetic recombination, are at least in part, independent processes and are related to the dark repair mechanisms. Moreover, not only mutants sensitive to both, ultraviolet light and ionizing radiation, but also the mutants sensitive to ionizing radiation alone, may affect the uv-induced mutability. It appears that in N. crassa sensitivity to gamma-rays interferes more readily with uv-mutagenesis when compared with the effect of sensitivity to ultraviolet light on uv-mutagenesis. A similar conclusion is implicit from the work of Baptist et al,, (1971) which led them to suggest that in E. coli factors enhancing gamma-sensitivity but without an effect on uv-inactivation of cells, interfere with the post-replication repair mechanism which is involved in uv-mutagenesis.

As stated earlier, the frequency of spontaneous as well as uv-induced mutations for caffeine resistance is considerably reduced in all gamma-ray sensitive mutants when compared to the wild type strain. This observation leads to the assumption that the wild type strain, pe (Y8743m) carries a mutation of a gene which controls mutability and consequently enhances the spontaneous as well as the uv-induced mutation rate in this strain. Hence, in its phenotypic response of mutability the wild type strain may be considered a mutator strain similar to those described in S. cerevisiae (von Borstel et.al.,1968). Based upon this argument it may be suggested that the six gamma-ray sensitive strains as described in this study, represent the mutations of a gene or genes, which besides eliminating the mutator activity present in their wild type progenitor, also block the process involved in the repair of the damage incurred from exposure to gamma radiation.

Due to the limited number of studies involving Eukaryotes, it is not possible at present to draw any definite conclusion regarding the inter-relationship of the processes of DNA repair, uv-mutagenesis and genetic recombination. Nevertheless, the result of the present investigation together with other studies on Eukaryotes (Table IX), have revealed that the genes controlling uv-mutagenesis do not affect genetic recombination. This is in contrast to findings in E. coli which indicate that both processes, genetic recombination and uv-mutagenesis, are controlled by the same genes (Witkin, 1969). Similarly, in yeast there are genes mutations of which reduce genetic recombination without altering the radiation sensitivity of the cells (Rodarte-Ramon and Mortimer, 1972).

The present results seem to lend support to the assumption that each of the three processes, genetic recombination, uv-mutagenesis and DNA repair after radiation damage, involves several steps and only a few of these steps are shared by all the pathways. Lemontt (1971b) has recently presented an evidence which suggests that in yeast, more than one pathway is involved in uv-mutagenesis e.g., strains carrying two rev mutations (defective in uv-mutability) are more uv-stable than single mutant strains. Interestingly, some observations made on Prokaryotes are also consistent with this hypothesis. For instance, not all recombination deficient mutants of Pseudomonas aeruginosa and of E. coli are sensitive to radiation (Holloway, 1966; Storm et al 1971). Also, Miura and Tomizawa (1970) showed that in bacteriophage lambda, mutants deficient in the recombination process behave in a normal fashion with regard to uv-mutability.

Although it can be assumed that the underlying mechanisms of DNA repair, uv-mutagenesis and genetic recombination are basically similar in both, Prokaryotes and Eukaryotes, it can also be expected

that these processes may have been variously modified in their degree and nature of interdependence as a result of evolutionary diversification. Therefore, the variable response of mutants defective in the dark repair processes, genetic recombination as well as in uv-mutagenesis, are probably manifestations of such modifications. In the light of the findings that mutations of genes controlling radiation sensitivity in yeast have specific effects on mitotic or meiotic (gene conversion or crossing over) recombinations (Rodarte-Ramon, 1972), it may be suggested that mutants defective in different types of genetic recombinations should be separately characterized for their response to uv-mutability of different loci (carrying known molecular types of mutations) and for radiation sensitivity.

Results of such investigations may elucidate the understanding of the mechanisms of DNA repair, uv-mutagenesis and genetic recombination and may also serve as the basis for an evaluation of the interdependence of these processes in both, Prokaryotes and Eukaryotes. Biochemical support to these investigations may be provided by the development of the techniques as used by Unrau et al.,(1971) to detect the radiation induced lesions in the DNA of S. cerevisiae.

## SUMMARY AND CONCLUSIONS

Using ultraviolet light as a mutagen (wavelength 2530 Å), six gamma-ray sensitive mutants have been isolated in Neurospora crassa. In addition, one of these mutants, gs-6, was found to be sensitive to uv-light. Tetrad analysis indicated that radiation sensitivity of these mutant strains is under the control of single nuclear genes. When induced with uv-light, all gamma-ray sensitive mutants yielded fewer mutations for caffeine resistance when compared to the wild type at equal levels of uv-dose or survival. Moreover, mutants gs-4 and gs-6 showed reduced mutability for adenine revertants ($ad^+$).

Crosses homozygous for each of the mutations, gs-2, gs-4, gs-6 and gs-20 were fertile and yielded normal frequencies of interallelic recombination (measured as $pan^+$ prototroph frequency) between two pan-2 alleles, pan-2(B3) and pan-2(B5).

The results of this investigation suggest that the process of uv-mutagenesis is interrelated with the DNA repair processes involved in increasing the survival after irradiation and is at least partly independent of the process of genetic recombination.

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